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Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 28.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Support: EMBO ALTF 303-2010

NIH NS081297

NIH MH095147

Title: Clonally related interneurons disperse both within and across functional boundaries within the forebrain

Authors: *C. MAYER[1], X. H. JAGLIN[1], C. L. CEPKO[2], S. HIPPENMEYER[3], G. FISHELL[1]; [1]Physiol. & Neurosci., NYU Neurosci. Inst., New York, NY; [2]Harvard Med. Sch., Departments of Genet. and Ophthalmology and HHMI, Boston, MA; [3]IST Austria, Klosterneuburg, Austria

Abstract: The medial ganglionic eminence (MGE) gives rise to the majority of mouse forebrain interneurons. Here, we examine the lineage relationships among MGE-derived interneurons using a replication-defective retroviral library containing a highly diverse set of DNA barcodes. Recovering the barcodes from the mature progeny of infected progenitor cells enabled us to unambiguously determine their respective lineal relationships. We found that clonal dispersion occurs across large areas of the brain and is not restricted by anatomical divisions. As such, sibling interneurons can populate the cortex, hippocampus and striatum. Importantly, we also revealed that the majority of interneurons were generated from asymmetric divisions of MGE progenitor cells, followed by symmetric divisions within the subventricular zone. Altogether, our findings uncover that lineage relationships do not appear to determine interneuron allocation to particular regions. As such, it is likely that clonally-related interneurons have considerable flexibility as to the particular forebrain circuits to which they can contribute.

Disclosures: C. Mayer: None. **X.H. Jaglin:** None. **C.L. Cepko:** None. **S. Hippenmeyer:** None. **G. Fishell:** None.

Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 28.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: P30 EY12196

Title: Wide dispersion and diversity of clonally related inhibitory neurons

Authors: *C. C. HARWELL;
Neurobio., Harvard Med. Sch., Boston, MA

Abstract: A majority of GABAergic forebrain neurons are derived from the medial ganglionic eminence (MGE).MGE progenitors predominantly give rise to two functionally distinct subtypes of interneurons: parvalbumin (PV)-expressing fast spiking interneurons and somatostatin-expressing non-fast-spiking interneurons. MGE progenitors are molecularly defined by the expression of the homeodomain transcription factor Nkx2.1, which is required for the migration and specification of mature interneuron cell identities. Once cells have achieved their terminal division they migrate long distances to reach their final destination in the developing brain. The extrinsic signals involved in regulating the migration and positioning of newborn neurons have been extensively studied. However, the roles of intrinsic determinants, such as clonal lineage, and their relationships to the distribution of MGE derived interneurons remains poorly understood. Utilizing lineage-specific retroviral barcode labeling, we find that clonally related interneurons can be widely dispersed throughout the forebrain, and are composed of both parvalbumin and somatostatin interneuron subtypes. These data suggest that distinct functional interneuron subtypes can be derived from the same clonal lineage, and their distribution in distinct forebrain structures is not driven by their clonal relationships.

Disclosures: C.C. Harwell: None.

Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH/NINDS RO1NS062849

NIH/NINDS R01NS078164

Title: Rewiring local inhibitory microcircuits by direct lineage reprogramming of neocortical projection neurons

Authors: *M. A. MOSTAJO RADJI[1], Z. YE[2], J. R. BROWN[3], C. ROUAUX[3], G. S. TOMASSY[3], T. K. HENSCH[2], P. ARLOTTA[3];
[2]Dept. of Mol. and Cell. Biol., [3]Dept. of Stem Cell and Regenerative Biol., [1]Harvard Univ., Cambridge, MA

Abstract: We have previously demonstrated that overexpression of the master transcription factor Fezf2 is sufficient to reprogram postmitotic callosal projection neurons (CPNs) of cortical layers 2/3 to a corticofugal projection neuron (CFuPN) identity within a defined window of time. The reprogrammed neurons acquire molecular properties of CFuPNs and change their axonal connectivity from interhemispheric, intracortical projections to corticofugal projections directed below the cortex. Here, we sought to determine whether reprogramming projection neuron identity is sufficient to instruct the remodeling of their surrounding local inhibitory circuits. We first investigated the extent of reprogramming by performing single cell gene expression analysis of reprogrammed neurons. Our data showed that a subset of Fezf2-overexpressing cells significantly upregulated multiple molecular markers of CFuPNs compared to control CPNs. In agreement, we find that iCFuPNs acquire electrophysiological properties that are typical of CFuPNs. Notably, recordings of miniature inhibitory postsynaptic currents (mIPSCs) demonstrated that iCFuPNs receive increased inhibitory inputs, which resembled those of deep layer CFuPNs. In order to gain insight into this electrophysiological adaptation, we studied more specifically the contribution of Parvalbumin (PV) positive interneurons (INs). We found that PV positive INs form more perisomatic synapses onto reprogrammed neurons than onto CPNs, with levels indistinguishable from endogenous CFuPNs in layer 5. Optogenetic recordings and immunohistochemical analysis further confirmed an increased number of synapses by PV INs onto reprogrammed neurons. Furthermore, we demonstrate that reprogrammed projection neurons are not intrinsically more sensitive to inhibition and therefore the observed increase of inhibitory input likely reflects rewiring of the cortical circuit, rather than the expression of more GABA receptors. Altogether, our results indicate that reprogramming the identity of projection neurons is sufficient to reshape the local inhibitory circuitry and highlights the importance of projection neuron diversity in controlling this process.

Disclosures: M.A. Mostajo Radji: None. **Z. Ye:** None. **J.R. Brown:** None. **C. Rouaux:** None. **G.S. Tomassy:** None. **T.K. Hensch:** None. **P. Arlotta:** None.

Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

Location: Hall A

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Program#/Poster#: 28.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Support: 5F30MH102002-02

Title: Genetic fate mapping of neocortical chandelier cell specification

Authors: *S. M. KELLY[1,2], M. MOISSIDIS[1], M. HE[1], Y. KIM[1], Z. HUANG[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Stony Brook Univ., Stony Brook, NY

Abstract: The diversity of GABAergic interneurons reflects a division of labor in regulating the balance, flexibility, and dynamic operations of cortical circuits, but the developmental processes that generate these diverse cell types are not well understood. Previous studies have identified embryonic neurogenic regions, such as the medial and caudal ganglionic eminence (MGE and CGE), which generate broad cohorts of GABA populations, but the progenitor mechanisms that give rise to distinct cell types remain unclear. In particular, it is unknown whether multipotent progenitors progressively generate multiple cell types or fate-restricted progenitors generate individual cell types. The chandelier cell (ChC) is one of the most distinctive GABA neuron types due to its axo-axonic synapses innervating the axon initial segment of pyramidal neurons, and thus provides a more tractable experimental system for the investigation of cell type specification. Here, we have taken an intersectional genetic approach to further define the progenitor types that give rise to ChCs and understand their temporal progression throughout embryogenesis. By combining Nkx2.1-FLP and Ascl1- or Dlx1- CreER drivers with a dual reporter, we have separately interrogated radial glia/apical progenitors (RGCs) and two types of intermediate/basal progenitor (IPC) within the MGE ($Nkx2.1^+/Asc1^+$ or $Nkx2.1^+/Dlx1^+$). This approach has revealed the birth pattern and progenitor origin of the laminar subtypes of ChCs, showing that unlike most MGE-derived interneurons, ChC production and laminar deployment do not follow an inside-out pattern: both L2 and L5/L6 ChCs are generated concurrently from mid- to late-gestation. Surprisingly, we found that $Ascl1^+$ RGCs are specified for production of L2 ChCs as early as E10, remaining latent for several days before completing the first wave of ChC neurogenesis (at ~E12). Subsequently, $Ascl1^-/Nkx2.1^+$ RGCs appear to produce the vast majority of L5 and L6 ChCs, while $Ascl1^+/Nkx2.1^+$ IPCs continue to produce L2 ChCs. Finally, at late embryonic timepoints, a pool of Ascl1+ intermediate progenitors becomes restricted for production of a burst of ChC between E16 and E18, which ultimately localize to all cortical layers and are particularly concentrated to medial prefrontal cortex. In contrast, $Nkx2.1^+/Dlx1^+$

IPCs generate few ChCs throughout the same period. Together these results implicate the role of fate-restricted progenitors in the generation of distinct cortical GABA interneurons.

Disclosures: **S.M. Kelly:** None. **M. Moissidis:** None. **M. He:** None. **Y. Kim:** None. **Z. Huang:** None.

Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 28.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Title: Study on lineage-specific gabaergic interneurons with brainbow vectors

Authors: ***M. WANG**, Y. H. FU, Y. C. YU;
Inst. of Brain Science, Fudan Univ., Shanghai, China

Abstract: Unlike simplex neocortical excitatory neurons, neocortical interneurons are various in types. Although the embryonic origins of diversity of interneurons have been examined in previous studies, little is known about the lineage development of interneurons at the single progenitor cell level, which would help explain the origin of multiple interneuron types. The traditional method to track lineage-specific neurons, low-titer injections of conditional reporter retroviral stocks, is hard to track single clone precisely or track different clones simultaneously. In order to solve the problem, we introduced a new method to label interneuron progenitors by electroporating the Brainbow vectors in Nkx2.1-creER mouse embryos. We found that the method help yield a sparse mosaic of color combinations in different clonal interneurons in MGE and POA of embryonic mice. Moreover, interneuron clones also could be identifiable on the basis of specific combinations of cytoplasmic and nuclear colors (up to 53 types) in adult stage, which make it more precise to label diversity of clones. In a word, it would be a great facility to study the clonal interneurons.

Disclosures: **M. Wang:** None. **Y.H. Fu:** None. **Y.C. Yu:** None.

Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH NINDS RO1NS085081

Title: T-box transcription factor Tbr2 controls multiple aspects of cortical projection neuron differentiation

Authors: *A. MIHALAS[1], R. DAZA[1], K. RAMOS[2], E. YOUNG[3], R. HEVNER[4];
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Abstract: Cerebral cortex excitatory projection neurons are born from a common radial glial progenitor (RGP). RGPs produce neurons either directly, or indirectly, via intermediate progenitors (IPs), a process named indirect neurogenesis. IPs are thought to be essential for the evolutionary enlargement of the cortex in primates, where there is a great expansion of IP population. Tbr2 is a well-established marker for IPs. Tbr2-deficiency in humans is associated with developmental defects such as microcephaly, motor and cognitive delays. Initially, IPs were hypothesized to mainly produce late born, upper layer neurons. Consistent with this hypothesis, previous Tbr2-mutant models have shown reduced IPs, with a consequent decrease of upper layer projection neurons, but no detectable changes in deep layer neurons. Recent findings hint that IPs produce all cortical layer neurons. This study aims to clarify the laminar and molecular fate identity of IPs. Using a combination of Tbr2-CreER allele and Ai14-reporter we permanently labeled cohorts of IPs and their progeny systematically from the beginning until the end of neurogenesis (mouse embryonic day E11.5 to E16.5). Overall, our results indicate that IPs contribute to all layers and produce all types of cortical excitatory neurons, including Cajal-Retzius and subplate neurons. Unexpectedly, we find that IPs in the early cortex (E12.5) produce not only deep but also superficial layers, indicating that some early IPs are fated to undergo prolonged proliferation and produce upper layer neurons. Interestingly, laminar differentiation of Tbr2-deficient IPs is perturbed. In contrast to previous reports, we find increased abundance of IPs in Tbr2-mutant cortex; differentiation of these IPs is delayed and prolonged in the intermediate zone. The laminar cytoarchitecture of Tbr2-deficient cortex appears to be maintained, but contrary to previous reports, we find a large expansion of Ctip2+ layer 5, at the expense of Cux1+ upper and Tbr1+ deep layers. These changes in laminar identity are accompanied by severe dysregulation of molecular expression in Tbr2-deficient cortex, and are consistent with an initial burst of early-born cells, followed by a gradual decrease in mid and late-born cells in Tbr2-mutant cortex. Mechanistically, our data suggests that in the absence of Tbr2, IPs express aberrant levels of pro-IP transcription factors Pax6 and Insm1, and neuronal committed progenitor marker NeuroD, in an effort to compensate for the neuronal differentiation

defects. In all, our data provide support that Tbr2 is crucial for correct specification of laminar and molecular projection neuron identity in the cerebral cortex.

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Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

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Topic: A.01. Neurogenesis and Gliogenesis

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Title: Excessive Wnt/beta-catenin signaling promotes midbrain floor plate neurogenesis, but results in vacillating dopamine progenitors

Authors: *N. NOURI[1], M. PATEL[3], M. JOKSIMOVIC[4], J.-F. POULIN[1], A. ANDEREGG[1], M. M. TAKETO[5], Y.-C. MA[2], R. AWATRAMANI[1];
[1]Neurol., [2]Pediatrics, Northwestern Univ., Chicago, IL; [3]Univ. of Chicago, Chicago, IL; [4]Med. Col. of Wisconsin, Milwaukee, WI; [5]Kyoto Univ., Kyoto, Japan

Abstract: The most debilitating motor symptoms of Parkinson's Disease (PD) result from the excessive loss of midbrain dopamine (mDA) neurons in the substantia nigra pars compacta, that transmit dopamine to the striatum. Recent protocols to convert human embryonic stem cells (hESC) or induced pluripotent stem cells (iPSC) to DA neurons have been generated for potential cell replacement therapeutics in PD patients and for PD modeling. However, further *in vivo* analysis of mDA specification is still needed to optimize these protocols for proper DA

neuron specification and differentiation. The floor plate (FP), a ventral midline structure of the developing neural tube, has differential neurogenic capabilities along the anterior-posterior axis. The midbrain FP, unlike the hindbrain and spinal cord floor plate, is highly neurogenic and produces mDA neurons. Canonical Wnt/beta-catenin signaling, at least in part, is thought to account for the difference in neurogenic capability. Removal of beta-catenin results in mDA progenitor specification defects as well as a profound reduction of neurogenesis. To examine the effects of excessive Wnt/beta-catenin signaling on mDA specification and neurogenesis, we have analyzed a model wherein beta-catenin is conditionally stabilized in the Shh+ domain. Here, we show that the Foxa2+/Lmx1a+ domain is extended rostrally in mutant embryos, suggesting that canonical Wnt/beta-catenin signaling can drive FP expansion along the rostrocaudal axis. Although excess canonical Wnt/beta-catenin signaling generally promotes neurogenesis at midbrain levels, less tyrosine hydroxylase (Th)+, mDA neurons are generated. This is likely because of improper progenitor specification. Excess canonical Wnt/beta-catenin signaling causes downregulation of net Lmx1b, Shh and Foxa2 levels in mDA progenitors. Moreover, these progenitors assume a mixed identity to that of Lmx1a+/Lmx1b+/Nkx6-1+/Neurog1+ progenitors. We also show by lineage tracing analysis that normally, Neurog1+ progenitors predominantly give rise to Pou4f1+ neurons, but not Th+ neurons. Accordingly, in the mutant embryos, Neurog1+ progenitors at the midline generate ectopic Pou4f1+ neurons at the expense of Th+ mDA neurons. Our study suggests that an optimal dose of Wnt/beta-catenin signaling is critical for proper establishment of the mDA progenitor character. Our findings will impact protocols that utilize Wnt pathway reagents to derive mDA neuron models and therapeutics for Parkinson's disease.

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Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 28.08/A8

Topic: A.01. Neurogenesis and Gliogenesis

Title: Single cell rna sequencing uncovers close relationship between dopamine and subthalamic nucleus neuron lineages

Authors: ***N. KEE**[1], L. DAHL[2], E. JOOMARDI[2], N. VOLAKAKIS[2], L. GILLBERG[2], Å. BJÖKLUND[3], H. STORVALL[2], R. SANDBERG[2], T. PERLMANN[2];

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Abstract: The extensive neuronal diversity generated from neural stem cells in the mammalian CNS is an impressive feat of development, and also presents a significant challenge to dissect and understand. Ventral midbrain (vMB) dopaminergic neurons are a neuronal subtype that have been rigorously studied, strongly motivated by the interest in engineering stem cells into dopamine neurons for cell replacement in Parkinson's disease. However, classical approaches used to probe vMB neuron diversity and development are hampered by the presence of mixtures of different cell types and maturation stages in whole tissue dissections. To resolve this limitation, we have utilized single-cell RNA-sequencing to reconstruct the differentiation of neuronal lineages in the mouse vMB between embryonic day 10.5 and 13.5. Importantly, the analysis provided a robust genome-wide reconstruction of how neural stem cells expressing the transcription factor Lmx1a transition into postmitotic differentiating neurons. This approach also allowed rapid, comprehensive and unbiased identification of robust gene signatures represented in Lmx1a-expressing neuronal lineages. *In vivo* validation of one such signature has uncovered an unexpected similarity between developing dopaminergic neurons, and more rostrally developing glutamatergic neurons of the Subthalamic Nucleus. Single-cell RNA-seq thus proves an invaluable technique in successfully interrogating lineage diversity in the developing CNS.

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Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Support: ANR grant ReSiNEs, ANR-11-BSV2-0003

LabEx ANR-10-LABX-0030-INRT

Title: Role of retinoic acid receptor beta in development and homeostasis of mouse striatum

Authors: *W. KREZEL, M. RATAJ-BANIOWSKA, A. NIEWIADOMSKA-CIMICKA, A. PODLESNY-DRABINIOK, T. YE, D. DEMBELE, P. DOLLÉ;
IGBMC, ILLKIRCH, France

Abstract: Over the recent decade, retinoid signaling emerged as an important regulator of development and functions of the basal ganglia (Krezel et al. 1999; Maden 2001; Liao et al. 2005; Krzyzosiak et al. 2010), but to date little is known about the mechanisms underlying such activities, namely its genomic targets and the neuroanatomical pathways (striatonigral and/or striatopallidal output pathways) it may regulate. Signaling by retinoic acid (RA), an oxidative derivative and active form of vitamin A (retinol), is mediated by binding to RA receptors (RARα, β, γ), which form heterodimers with retinoid X receptors (RXRα, β, γ) and act as ligand-controlled transcription factors. Several lines of evidence indicate that RA is particularly important for striatal functions and pathology. Among different brain regions, the adult rodent striatum contains some of the highest levels of RA (Kane et al. 2005). The striatum is also a site of strong expression of two retinoid receptors, RARβ and RXRγ (Zetterstrom et al. 1994; Krezel et al. 1999). Genetic ablation of RARβ and/or RXRγ leads to abnormal striatal functions, revealed by deficits in motor coordination and depressive-like behaviors (Krezel et al. 1998; Krzyzosiak et al. 2010). Whereas some of these phenotypic abnormalities may have a developmental origin, post-natal (physiological) functions of these receptors have also been demonstrated (Krzyzosiak et al. 2010; Wietrzych-Schindler et al. 2011). We will present data from mice carrying null mutation of RARb receptor supporting role of RARb in control of development of striatonigral dopaminergic pathway through mechniams which may involve FGF and MEIS signaling. Deficits in the number of DRD1-positive striatonigral neurons may result from premature differentiation of progenitor cells, leading also to an overall reduction of proliferation index reported also previously by Liao and colleagues (Liao et al. 2005). In addition to its prenatal functions, RARb may also control a number of cell functions in the striatum as indicated by ChIP-seq, genome-wide identification of RARb binding sites and transcriptomic analyses of RARb-null mutant striatum, which will be presented. Such regulations may have direct relevance for pathophysiology of Huntington's disease.

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Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Hong Kong University of Science and Technology (R9321)

Hong Kong RGC (GRF 660813)

Title: The clonal organization of the mammalian CNS: numerical quanta of cerebellar cells define highly specified developmental lineage relationships

Authors: *K. HERRUP[1], K.-H. TSE[2], K. K. Y. CHAN[2], K. NEVES[3], H.-M. CHOW[2,4], S. HERCULANO-HOUZEL[3];
[1]Hong Kong Univ. of Sci. & Technol., Kowloon, Hong Kong; [2]Div. of Life Sci., Hong Kong Univ. of Sci. & Technol., Clear Water Bay, Kowloon, Hong Kong; [3]Inst. de Ciências Biomédicas, Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [4]Inst. for Advanced Studies, Clear Water Bay, Hong Kong

Abstract: Developmental neurobiologists have defined how patterns of cell divisions in the ventricular zone change over time and how they help to specify the fate of daughter cells. So far, however, what factors control the number of cells that are generated remains a mystery. For any given lineage of cells, this numerical control could be intrinsic to the lineage itself or extrinsic (influenced by cells from other lineages). Early studies of mouse aggregation chimeras suggested a prominent role for intrinsic, lineage-autonomous regulation. The suggestion was that a small number of progenitor cells, selected early in development, serve as the sole source of an entire neuronal population (e.g., Purkinje cells, or facial nucleus neurons). A key feature of the model is that these progenitors, once specified, each go through the same specific number of cell divisions thus producing a quantum of cells. This hypothesis predicts that in normal wild type mice, quantitative variations in the determination of the number of the early progenitors should lead to large quantal variations in the total number of descendent cells. Thus, counts performed in a large enough number of brains should reveal a distribution that is not Gaussian, but rather multimodal, with each mode representing the addition or subtraction of a single quantum of cells. We report here that this prediction is born out. Using the isotropic fractionator to achieve high-throughput and quantitative reproducibility, we have counted the number of cells in 174 half cerebella from 47 males and 46 female C57BL/6J adult animals of similar age. The total number of cells in each hemisphere exhibits a multimodal distribution with peaks at 25, 28 and 31 million cells - consistent with the formation of each cerebellar half by 8, 9 or 10 quanta of slightly more than 3 million cells. We find no significant correlation between the numbers of cells in the left and right halves of the cerebellum across animals, which confirms earlier predictions that the progenitors of the left and right sides are specified independently. There is a significant bias towards a larger number of progenitors on the left side, and we observed a modest, but not significant ($p = 0.0686$) sexual dimorphism. In males the distribution is skewed towards the peaks at 28 and 31 million cells but towards the peaks at 25 and 28 million cells in females. The data confirm that the control of cell number in the mammalian CNS relies heavily

on intrinsic, lineage-based mechanisms. The use of genetically inbred, but otherwise normal animals to conduct this analysis allows the analysis of cells in a wide range of different brain regions.

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Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 28.11/A11

Topic: A.01. Neurogenesis and Gliogenesis

Title: Role of intermediate progenitors in the specification of cortical pyramidal neuron subtypes

Authors: ***J. M. LEVINE**[1,2,3], D. HUILGOL[1], Z. HUANG[1];
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Abstract: Pyramidal neurons (PyNs) comprise ~80% of cortical neurons, constituting a myriad of inter-areal processing streams and cortical output channels. The progenitor cells that give rise to PyNs mainly include radial glial cells (RGCs) and intermediate progenitor cells (IPCs) located in the embryonic cerebral ventricle wall. RGCs divide asymmetrically to generate neurons either directly or indirectly through IPCs, which divide symmetrically to produce pairs of PyNs. It remains unclear how progenitor types (e.g. RGCs, IPCs), their lineage progression, and timing of neurogenesis contribute to the specification of diverse PyN subtypes, as defined by axon projection. In particular, the role of IPCs in the generation of PyNs is poorly understood. We have generated an inducible Cre mouse driver line, which allows comprehensive lineage tracing from IPCs. We have further developed a novel genetic method to fate map neurons according to their birth time. We are fate mapping IPC-derived PyNs throughout embryogenesis. In addition to assessing the laminar position of PyN subtypes, axon projections are analyzed with viral labeling of fate-mapped PyNs. Our latest results will be presented.

Disclosures: **J.M. Levine:** None. **D. Huilgol:** None. **Z. Huang:** None.

Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

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Topic: A.01. Neurogenesis and Gliogenesis

Support: CIHR grant

VSRP fellowship

Title: GFAP+ and Oct4+ neural stem cells give rise to distinct neural progenitor cells in the perinatal mouse brain

Authors: *S. YAMMINE[1], J. GOSIO[2], D. VAN DER KOOY[1];
[1]Mol. Genet., [2]Human Biol., Univ. of Toronto, Toronto, ON, Canada

Abstract: There are at least two distinct types of neural stem cells (NSCs) in the developing mouse embryo that generate the diversity of neural progenitor cells (NPCs) that build the brain. Primitive NSCs form clonogenic neurospheres when grown in LIF and persist into the adult brain, where we have shown they express the pluripotency gene *Oct4* and give rise to the $GFAP^+$ definitive NSCs that form neurospheres in EGF and FGF2. We used the neurosphere assay *in vitro* to enrich for progenitor cells downstream either NSC type to characterize the functional differences between these two NSC populations. Here we show both NSCs derived from the E17.5 mouse brain give rise to many NPCs that are bipotent in the neuronal and glial lineages, though primitive NSCs give rise to more neuron-only NPCs and definitive NSCs give rise to many astrocyte-only NPCs. Most interesting, progenitor cells from either NSC that gave rise to both lineages generated significantly more progeny than those committed to either glial or neuronal fates. As primitive NSCs can give rise to definitive NSCs as well as neurons and glia, we asked whether primitive NSCs directly produce neurons and glia without a definitive NSC intermediate step. To test this, we grew spheres from early postnatal mice with herpes simplex virus thymidine kinase expression driven by the *GFAP* promoter, which causes *GFAP*-expressing cells (ie. definitive NSCs) to be killed upon division following administration of ganciclovir *in vitro*. There is a significant reduction in the number of neurospheres that form in EGF and FGF2 from definitive NSCs from the subependymal zone of early post-natal mice with this kill of *GFAP*-expressing cells, as expected. Results show primitive NSCs from these mice where *GFAP*-expressing cells were killed could still form $GFAP^+$ astrocytes, however the number of $BIII^+$ neurons was reduced. This suggests that astrocytes can be formed from progenitor cells that do not upregulate *GFAP* expression until after they are post-mitotic, and that neurons are made from mitotically active *GFAP*-expressing progenitors. By combining these data, we construct novel lineages for progenitor cells downstream primitive and definitive NSCs.

Disclosures: **S. Yammine:** None. **J. Gosio:** None. **D. van der Kooy:** None.

Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

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Åke Wiberg Foundation

Karolinska Institutet Research Foundations

Title: Single-cell and bulk RNA-Seq reveal distinct cell states within the oligodendrocyte lineage in the mouse brain

Authors: *G. CASTELO-BRANCO[1], S. MARQUES[1], A. ZEISEL[1], D. VANICHKINA[2], S. SAMUDYATA[1], A. MUNOZ MANCHADO[1], R. TAFT[2], J. HJERLING-LEFFLER[1], S. LINNARSSON[1];
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Abstract: Oligodendrocytes are glial cells that mediate myelination of neurons, a process that allows efficient electrical impulse transmission in the central nervous system. An autoimmune response against myelin triggers demyelination in multiple sclerosis (MS). Oligodendrocyte precursor cells (OPCs) can initially differentiate and promote remyelination in MS, but this process eventually fails in progressive MS. OPCs go through several cell states during development and disease, that ultimately define their potential to differentiate and myelinate. In order to clearly define distinct epigenetic states of OPCs and other oligodendrocyte lineage cells during development, we have performed single-cell and bulk RNA sequencing of cells of the oligodendrocyte lineage from mouse brain. We have found that FACS-sorted OPCs isolated from different locations in the central nervous system and developmental stages have distinct transcriptional profiles. Furthermore, by single-cell RNA-Seq of cells of the oligodendrocyte

lineage, we identified several sub-populations of cells, representing unique stages during the process of differentiation and myelination. We have further identified non-coding RNAs that are uniquely expressed in these distinct populations of the oligodendrocyte lineage. We are currently investigating if these non-coding RNAs play important roles in OPC differentiation and myelination.

Disclosures: G. Castelo-Branco: None. **S. Marques:** None. **A. Zeisel:** None. **D. Vanichkina:** None. **S. Samudyata:** None. **A. Munoz Manchado:** None. **R. Taft:** None. **J. Hjerling-Leffler:** None. **S. Linnarsson:** None.

Poster

028. Neurogenesis and Gliogenesis: Lineage and Cell Fate

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 28.14/A14

Topic: A.01. Neurogenesis and Gliogenesis

Support: Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, USA.

Title: Neurotrophic factor-α1 (NF-α1): A key factor for down-regulating neural stem cell proliferation and promoting differentiation to astrocytes

Authors: *P. SELVARAJ[1], S. MURTHY[1], C. LEE[1], M. LANE[2], N. CAWLEY[1], I. MERCHENTHALER[2], S. AHN[1], P. LOH[1];
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Abstract: Neurotrophic factor-α1 is a new neurotrophic factor shown to promote survival of embryonic hippocampal neurons. Our qRT-PCR (N=3) and *in situ* hybridization studies showed that NF-α1 mRNA is detectable as early as E5.5 in mouse embryos and increased significantly (p=0.01) in the brain from E10.5 to adulthood. Addition of recombinant NF-α1 (200nM) to E13.5 neocortex-derived neurospheres containing stem cells (5d culture), grown in the presence of FGF2 and EGF, reduced proliferation of the neurospheres by 37% (N=4; p=0.02). Since NF-α1 interacts with Wnt3a ligand binding receptor (Frizzled) and negatively regulates β-Catenin expression, we investigated whether β-Catenin and its downstream molecules known to regulate cell proliferation are involved. We found a 30% decrease in β-Catenin (N=3; p=0.01), 50-60%, decrease in Hes1 (N=3; p=0.01), and no change in CyclinD1 (N=3) protein levels in NF-α1 treated neurospheres compared to control, indicating that NF-α1 negatively regulates cell proliferation in neurospheres via Wnt signaling. Further, to assess the role of NF-α1 in neural

stem cell (NSC) differentiation, neurospheres from 7d cultures were dissociated and grown for 5d in the presence of NF-α1 and 1% FBS. Immunocytochemical analysis of differentiated cells labeled with specific antibodies against neurons (Tuj-1), astrocytes (anti-GFAP) and oligodendrocytes (anti-CNPase) showed a 17% (N=3; p=0.04) increase in GFAP+ in the presence of NF-α1, without altering the % of Tuj-1+ and CNPase + populations. Double immunostaining against nestin (anti-nestin) and astrocytes revealed increase of the GFAP+ population upon NF-α1 treatment comes from GFAP+ cells alone and not from intermediate glia population (GFAP+ nestin+, N=3; p=0.03). NF-α1-knockout & NF-α1-Wild type embryos were generated and their differentiation profile was checked. NF-α1-KO cells showed decrease in 15% GFAP+ cells (N=3; p=0.001) and increase in 18% Tuj-1+ cells (N=3; p=0.001), whereas it is opposite when compared to NF-α1-WT controls. This difference was rescued in NF-α1-KO cultures upon NF-α1 treatment. We also found NF-α1 significantly increased (N=3; p=0.01) phospho-ERK signaling and the downstream molecule Sox9 protein levels (N=3; p=0.01) which has been shown to be involved in astrocyte differentiation. Thus, we have uncovered an important factor, NF-α1 that modulates NSC differentiation into astrocytes.

Disclosures: P. Selvaraj: None. **S. Murthy:** None. **C. Lee:** None. **M. Lane:** None. **N. Cawley:** None. **I. Merchenthaler:** None. **S. Ahn:** None. **P. Loh:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.01/A15

Topic: A.04. Axon and Dendrite Development

Support: NIH Grant RO1 NS075156 (ZGZ)

Title: Exosomes derived from cerebral endothelial cells locally promote distal axonal growth

Authors: *Y. ZHANG[1], M. CHOPP[1,2], C. LI[1], X. LIU[1], X. WANG[1], L. ZHANG[1], Z. ZHANG[1]; [1]Neurol. Res., HENRY FORD HOSPITAL, Detroit, MI; [2]Dept. of Physics, OAKLAND UNIVERSITY, Rochester, MI

Abstract: Stroke induced angiogenesis promotes neuronal remodeling, and mechanisms underlying this neurovascular coupling have not been extensively studied. Exosomes are microvesicles which carry biological materials including RNAs and proteins, and play a pivotal role in the cell-cell communication. In the present study, we tested the hypothesis that exosomes derived from cerebral endothelial cells (CECs) promote distal axonal growth of cortical neurons.

Embryonic cortical neurons were cultured in a microfluidic culture device which separates somata and axons. Exosomes (3x107particles/ml) derived from primary cultured CECs of non-ischemic and ischemic rats were applied into the axonal compartment. During the 3 day culture, exosomes derived from non-ischemic CECs significantly increased distal axonal growth (24h, 528±24 μm; 48h 793±19 μm; 72h 1078±35 μm; $p<0.001$, n=3), compared to the axons of the non-exosome treatment group (24h, 378±10 μm; 48h, 567±14 μm; 72h 915±20 μm). Moreover, exosomes from ischemic CECs further increased axonal growth (24h, 611±23 μm; 48h 982±24 μm $p<0.001$, n=3) compared to exosomes from non-ischemic CECs. Confocal microscopic analysis showed that exosomes labeled with fluorescent siRNA internalized into the distal axons. Blockage of endocytosis with botulinum neurotoxin types A (BoNT/A) completely abolished exosome internalization and suppressed exosome-promoted axonal elongation, but did not induce cell death. We then examined whether internalized exosomes altered axonal miRNA profiles. Using miRNA PCR array, we found that exosomes contained abundant miRNAs and that 24 miRNAs in exosomes from ischemic CECs were significantly increased compared to exosomes from non-ischemic CECs. In the distal axons treated with exosomes from ischemic CECs, we detected significant increases in 8 out of these 24 miRNAs. One of the 8 increased miRNAs in the axon was miR-27a. Western blot analysis showed that Sema6A, one of verified targets of miR-27a, was significantly decreased in the axon treated with exosomes from ischemic CECs (0.3±0.1 vs 1 in exosomes from non-ischemic CECs, $p<0.05$, n=3). Using gain- and loss-of-function approach, we found that elevation and reduction of miR-27a in the distal axon by miR-27a mimics and inhibitors, respectively, increased and decreased axonal growth. Collectively, our data indicate that exosomes from CECs in particular from ischemic CECs robustly promote axonal growth, that the endocytosis process likely mediates exosomes internalization, and that miR-27a along with other miRNAs transferred by exosomes interact with their targets in the axons, leading to enhancement of axonal growth.

Disclosures: **Y. Zhang:** None. **M. Chopp:** None. **C. Li:** None. **X. Liu:** None. **X. Wang:** None. **L. Zhang:** None. **Z. Zhang:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.02/A16

Topic: A.04. Axon and Dendrite Development

Support: FONDECYT-1110723

CONICYT 21110539

Title: Expression pattern of guidance cues and extracellular matrix molecules in the prosomere 1 during posterior commissure development

Authors: *K. STANIC[1], N. SALDIVIA[1], M. GONZALEZ[1], B. FÖRSTERA[3], J. R. BENITEZ[2], H. MONTECINOS[1], T. CAPRILE[1];
[1]Axon Guidance Lab., [2]Enzymology Lab., Univ. De Concepcion, Concepcion, Chile; [3]Dept. of Physiol., Univ. de Concepcion, Concepcion, Chile

Abstract: During development of the central nervous system (CNS) it is crucial for the axons of projecting neurons to reach their target structures. The successful establishment of these connections is pivotal for any process that involve more than one area of the CNS and disruption of these pathways can cause substantial impairments in brain structure and functionality leading to congenital disorders, such as holoprosencephaly, horizontal gaze palsy, congenital mirror movements and congenital fibrosis of the extraocular muscles, among others. The mechanisms which lead to these disorders are still poorly understood how ever it is known that in most of these cases axon guidance molecules are involved. Furthermore, these mechanisms could also be important to aim regeneration therapies after nerve injury in the future. Controlled spatio-temporal expression of guidance molecules during development of the CNS is essential for the ability of growing axons to reach their proper targets. Never the less guidance cues are not sufficient to achieve this process. For a long time extracellular matrix molecules (ECM) have not gained much attention, however resent findings support the idea that they have a pivotal role during axon guidance. This work provides important information regarding possible molecular pathways that pretectal axons from the prosomere 1 follow along basal-alar plate in order to reach the CNS midline and which molecules might be helping in their decision to cross to the contralateral side in the diencephalic roof plate area, leading to the formation of the posterior commissure (PC). All experiments where performed in chick embryos and different stages of development where investigated. Patterning analysis was held by immunohistochemical techniques for the following molecules: SCO-spondin, EphA7, Tenascin, Laminin, Chondroitin sulphate, Osteopontin, HNK-1 and Fibronectin in the PC region. Transcript analysis was carried out for EphA7 by *in situ* hibrydization as well as RT-PCR for different sets of EphA-EprhinsA family members. To identify axonal receptors that bind to these molecules we performed immunocytochemistry in primary culture of pretectal neurons. The results of this study draw a map of the investigated molecules in the prosomere 1 letting us know the molecular expression pattern related with pretectal axons route, and provides important data to elucidate possible mechanisms of how this process occurs. The understanding of how guidance cues act under different environments can contribute to ultimately develop more concrete tools for axon regeneration.

Disclosures: **K. Stanic:** None. **N. Saldivia:** None. **M. Gonzalez:** None. **B. Förstera:** None. **J.R. Benitez:** None. **H. Montecinos:** None. **T. Caprile:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.03/A17

Topic: A.04. Axon and Dendrite Development

Title: Neurotropin abrogates lidocaine-induced suppression of neurite growth in cultured rat spinal neurons

Authors: *R. ISONAKA[1], A. TAKEUCHI[2], T. KATAKURA[1], K. HOSONO[1], T. KAWAKAMI[1];
[1]Dept. of Physiol., [2]Dept. of Med. Informatics, Kitasato Univ. Sch. of Med., Sagamihara, Japan

Abstract: Neuropathic pain is caused by primary lesions, dysfunction, or transient perturbation in the peripheral or central nervous system. Neuropathic pain is often chronic and analgesic agents are often used for treatment. Lidocaine relieves a variety of neuropathic pains but is reported to have neurotoxic effects. Neurotropin is an analgesic agent used for the treatment of various chronic pains and neuropathic pains. We hypothesized that neurotropin can provide neuroprotection and prevent neurotoxicity. To test our hypothesis, we examined the effects of neurotropin and lidocaine on neurite growth in cultured rat spinal neurons and how neurotropin interacts on lidocaine-induced neurotoxicity. Spinal neurons were identified by neurofilaments immunostaining and the length of the individual neurites of the neurons was measured using NIH Image software on the captured images. Neurotropin alone had no effect on neurite growth, whereas it protected against lidocaine-induced suppression of neurite growth in spinal neurons. These results suggested that neurotropin can protect against lidocaine-induced neurotoxicity.

Disclosures: R. Isonaka: None. **A. Takeuchi:** None. **T. Katakura:** None. **K. Hosono:** None. **T. Kawakami:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.04/A18

Topic: A.04. Axon and Dendrite Development

Title: Development of an automated growth cone collapse assay

Authors: *P. JOYCE[1], M. MANGAN[1], A. CURNOCK[1], S. MEISLER[2];
[1]Vertex Pharmaceuticals (Europe) Ltd, Abingdon, United Kingdom; [2]Vertex Pharmaceuticals Inc., Boston, MA

Abstract: Analysis of growth cone dynamics has proved to be invaluable tool for the identification of axonal repellent components, leading to an extensive understanding of axonal guidance pathways in neuronal development and repair. Compounds that are able to preserve growth cones and promote axonal growth have been proposed as therapeutics for a number of neurological diseases. Growth cone collapse assays are associated with labour intensive, manual image capture and analysis. Consequently, they are useful for identifying compounds that influence growth cone morphology but lack the throughput required to support drug discovery. A reproducible growth cone collapse assay that is amenable to compound screening is therefore highly desirable. We have developed an automated growth cone collapse assay, which includes robotic and reproducible neuron plating in 96 well plates, automated image capture and tailored image analysis of growth cones. The assay was developed using primary rodent motor neurons but is adaptable to other neuronal cell types, and is responsive to multiple axonal repellent molecules. Importantly, the assay provides a quantitative measure of compound potency for inhibition of axonal repellent driven growth cone collapse.

Disclosures: **P. Joyce:** A. Employment/Salary (full or part-time):; Vertex Pharmaceuticals Europe Ltd. **M. Mangan:** A. Employment/Salary (full or part-time):; Vertex Pharmaceuticals Europe Ltd. **A. Curnock:** A. Employment/Salary (full or part-time):; Vertex Pharmaceuticals Europe Ltd. **S. Meisler:** A. Employment/Salary (full or part-time):; Vertex Pharmaceuticals Incorporated.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.05/A19

Topic: A.04. Axon and Dendrite Development

Title: Nerve growth factor (NGF) facilitates innervation of perivascular nerves in tumor neovasculatures of mouse corneal

Authors: *H. KAWASAKI[1], S. TAKATORI[2], Y. SONE[3], E. OCHI[3], A. MATSUYAMA[3], M. GODA[3];
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Abstract: Background: The vascular tone and tissue blood flow are maintained and regulated by perivascular nerves. However, many studies have reported that tumor neovascular vessels have no innervation of perivascular nerves. We have shown that nerve growth factor (NGF) facilitated perivascular innervation and suppressed the tumor growth (1). From these results, we hypothesized that the neuronal regulation of blood flow toward tumors via perivascular nerves may lead suppression of the tumor growth. Therefore, the aim of this study is to investigate effect of NGF on distribution of perivascular nerves and neovessel form in tumor tissues, which were generated by mouse corneal micropocket method. Methods: Under pentobarbital anesthesia, a small incision was made in the center of eye ball. A gel, which contained DU145 prostate carcinoma cells or HT 1080 fibrosarcoma cells, was implanted into the mouse corneal micropocket. NGF or saline was subcutaneously administered using an osmotic mini-pump implanted the dorsal area. After 1 week, the distribution of perivascular nerves in mouse corneal were immunohistochemically studied. Also, the density of neovessels (immunocytochemically stained CD31) and smooth muscles (α-smooth muscle actin; SMA) in tumor tissues was quantified by the computer-assisted image processing. Results: Four days after implantation of tumor cells in mouse corneal, many neovessels generated from corneal limbal vessels were observed in DU145 and HT1080 tumor tissues. The bleedings around DU145 and HT1080 tumor tissues were observed in saline-treated control, while no bleeding was not found in tumor tissues of NGF-treated corneal. Treatment of mouse with NGF resulted in innervation of perivascular nerves around tumor neovessels, but not observed in saline-treated group. NGF treatment caused significant increase in SMA-, but not CD31-, immunopositive cells. Conclusion: These results suggest that NGF may facilitate innervations of perivascular nerve to regulate the blood flow in tumor neovessels. Reference: (1) Goda M et al.: Nerve growth factor suppresses prostate tumor growth. J Pharmacol Sci, 112: 463-466 (2010)

Disclosures: H. Kawasaki: None. **S. Takatori:** None. **Y. Sone:** None. **E. Ochi:** None. **A. Matsuyama:** None. **M. Goda:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.06/A20

Topic: A.04. Axon and Dendrite Development

Support: Conacyt CB09/131281

Papiit IN208713

Title: Semaphorin 3C released by a biocompatible hydrogel guides and promotes axonal growth of rodent and human dopaminergic neurons

Authors: *O. A. CARBALLO MOLINA[1], A. SÁNCHEZ-NAVARRO[1], A. LÓPEZ-ORNELAS[1], A. CAMPOS-ROMO[2], I. VELASCO[1];
[1]UNAM, Distrito Federal, Mexico; [2]Periférica de Neurociencias de la Facultad de Medicina - UNAM en el Inst. Nacional de Neurología y Neurocirugía, Distrito Federal, Mexico

Abstract: The restoration of dopaminergic circuits could increase the effectiveness of the cell replacement therapy in Parkinson Disease (PD), in which dopamine neurons die. To achieve this goal, guidance of dopaminergic axonal (DAx) growth is desirable. Semaphorin 3C (Sema3C) is a molecule that participates in the formation of dopaminergic circuits during development. Sema3C attracts and causes enhanced DAx growth of rodent cells both *in vitro* and *in vivo*. In this work we used Puramatrix, a polypeptide hydrogel (Hyd) that polymerize at physiological ionic concentration and is compatible with the nervous tissue, as a protein carrier for Sema3C. We found that this Hyd is able to incorporate Sema3C and release it to the medium, after quantification by ELISA. We evaluated if Sema3C delivery by Hyd attracts DAx growth: cultured midbrain explants (MbE) of rat embryos were placed into collagen gel matrices, to allow axonal growth in all directions. MbE were placed close to explants of pretectum (PT), which is known to exert an attractive effect over DAx growth. In parallel experiments, MbE were exposed to Hyd coupled to Sema3C. We found that Sema3C released by Hyd induced an attractive effect over DAx growth similar to the PT explants, which was not observed with Hyd containing a control protein. To quantify the attractive effects, we used an index that was obtained by dividing the average DAx length of the proximal by the distal growth. We also analyzed if Sema3C released by Hyd was able to promote DAx growth using microfluidic devices, using cultured dissociated rat dopaminergic neurons or dopaminergic neurons differentiated from human embryonic stem cells. These devices contain microgrooves that are transversed by the growing axons in a linear trajectory, making easy to measure axonal length. The DAx growth of both rat and human dopamine neurons increased in response to soluble Sema3C, and also to Sema3C released by the Hyd. These results show that this Hyd is able to incorporate and release Sema3C, and such delivery guides and promotes DAx growth. This delivery system might be used as a Sema3C carrier for *in vivo* studies in PD animal models.

Disclosures: O.A. Carballo Molina: None. **A. Sánchez-Navarro:** None. **A. López-Ornelas:** None. **A. Campos-Romo:** None. **I. Velasco:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.07/A21

Topic: A.04. Axon and Dendrite Development

Title: Reaching new distances: extending neuronal regeneration with nanogrooves

Authors: *M. FORNARO[1], C. SIGERSON[1], H. SHARTHIYA[1], C. DIPOLLINA[2], D. GIAMBALVO[2], J. GASIOROWSKI[2];
[1]Dept of Anat., [2]Biomed. Sci., Midwestern Univ., Downers Grove, IL

Abstract: Peripheral nerve damage is a common result of many injuries and disease states. While the peripheral nervous system regenerates spontaneously, the extent to which it does is often insufficient to fully restore function. Such a loss can have a severe impact on quality of life for many patients. There is a great deal of research in increasing axonal growth using biochemical growth factors. While these growth factors may increase axonal development, it is often uncontrolled and random in direction. Such growth is inefficient in delivery of neurites to more distant structures. Therefore, the aim of this study is to control the direction of axonal growth using nano- to micron scale grooves as topographical cues. We believe organized and direct axonal growth will increase the effective reach of these neurites and could lead to a more functional recovery. Our hypothesis is that axons will use the biophysical signals as a guide and propagate parallel to them which will lead to a longer effective axonal length. For the purposes of this study, we used *ex vivo* explants of mouse dorsal root ganglia (DRGs) as our experimental model. Cervical and thoracic DRGs were harvested and cultured on varying sizes of anisotropic grooves. Three groove widths were tested: 200nm, 400nm, 700nm, and 2,000nm. A chemically identical flat surface was used as a control. All groups were maintained in Serum Free Medium (SFM) with 5ng/mL Nerve Growth Factor (NGF) for 6 days. They were then fixed, immunolabeled, and visualized using fluorescent confocal microscopy. Imaging and analysis demonstrated that axons align and grow in a linear fashion along the 700 and 2,000 nm grooves significantly more than the control. No significant difference was seen for the 200nm and 400nm grooves. This manner of direct propagation also significantly extended the average radius of growth of the axons in the 700 and 2,000 nm groups with a 50% increase in the average effective axonal length seen in the 700 nm group over the control. In conclusion, these results may translationally be applied toward novel treatments to directionally enhance spontaneous nerve regeneration across distances greater than those which occur naturally.

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Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.08/A22

Topic: A.04. Axon and Dendrite Development

Support: ERC StG#311159 - ZebraTectum

Title: Reelin control of retina ganglion cells synaptic lamination

Authors: ***F. DEL BENE**[1], T. O. AUER[1], N. TESTA[1], J.-P. CONCORDET[2], V. DI DONATO[1]; [1]Inst. Curie - Ctr. de Recherche, Paris, France; [2]INSERM U1154, CNRS UMR7196, MNHN USM503, Muséum Natl. d'Histoire Naturelle, Paris, France

Abstract: Neuronal connections in the retina as well as in the retino-recipient nuclei are arranged in layers. Understanding the basis of class-specific targeting of axons to appropriate regions of the brain is critical to gain deeper insights on how complex neural networks form. In the retino-tectal circuit of zebrafish larval brain, the retinal ganglion cells (RGCs) convey the visual information projecting their axons to different layers in the optic tectum (OT). We found that a specific type of inhibitory interneurons, the Superficial Inhibitory Neurons (SINs), located at the surface of the tectal neuropil, express the extracellular matrix protein Reelin. During the development of the central nervous system (CNS), the secreted Reelin controls the migration and laminar arrangement of neurons in various structures including the neocortex, hippocampus, cerebellum and spinal cord. In mammalian visual system few studies have addressed its role in axonal targeting. Here, we show that a gradient of Reelin is established along the neuropil, likely due to secretion by the SINs. TALENs- and CRISPRs-mediated gene disruption for reelin and dab1 (the intracellular mediator of the signaling pathway) revealed aberrant layering of RGCs axons without affecting the overall retino-tectal projection pattern. Furthermore, transplantation experiments of wild type RGCs into a reelin mutant genetic background indicates that Reelin acts non cell-autonomously to ensure proper axonal lamination. Finally, we show that the signaling cascade in the OT might be driven by the very-low-density lipoprotein receptor (VLDLR). In fact, we demonstrated that VLDLR is the only described canonical Reelin receptor expressed in the ganglion cell layer of the zebrafish retina and we show that it is transported anterogradely to the axonal branches. All together our findings elucidate a new role for the Reelin pathway in vertebrate visual system development, during which it acts as molecular cue

by imparting lamina-specific positional information on the ingrowing axons of retinal ganglion cells.

Disclosures: F. Del Bene: None. **T.O. Auer:** None. **N. Testa:** None. **J. Concordet:** None. **V. Di Donato:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.09/A23

Topic: A.04. Axon and Dendrite Development

Support: NSF IGERT 0903622 NeuroEng

NSF IGERT CMMB 0965918

NSF CAREER DMR 0847253

NSF STC EBICS CBET 090939511

Title: Engineering a 3d platform to mimic *in vivo* neural network morphology and activity

Authors: *C. S. LIU[1], M. LEE[2], B. J. SLATER[3], G. NASERI KOUZEHGARANI[3], M. YU[4], O. V. CANGELLARIS[5], D. A. LLANO[3], H. KONG[2], M. U. GILLETTE[1];
[1]Cell and Developmental Biol., [2]Chem. and Biomolecular Engin., [3]Neurosci., [4]Mol. and Integrative Physiol., [5]Bioengineering, Univ. of Illinois, Urbana, IL

Abstract: Engineered 3D microenvironments are enabling the creation of designer neuronal networks that approximate the geometry of neuronal tissues. Here we use 3D alginate hydrogels functionalized with RGD (Arginine-Glutamate-Aspartate) peptides to direct the growth of neuronal processes and shape the formation of the developing networks. Through a glacier-moraine process of freezing-drying poly-lactic-co-glycolic acid (PLGA) microparticles, random and uniaxially aligned porous microchannels were created within the gels (Lee et al., Adv. Healthc. Mat., 2015). We grew early postnatal neurons from rat hippocampal and also adult rat dorsal root ganglion (DRG) neurons in these gels. Neurite processes were significantly more directed on the uniaxially aligned hydrogels compared to those grown in the random-pored gels. Using immunofluorescence imaging, we found that the morphologies of the neuronal networks grown in the uniaxial gels from hippocampal and DRG neurons are very similar to laminar neurite alignments in the CA1 region of the hippocampus as well as in nerves extending from

DRGs, respectively. To examine the functionality of the neuronal network, we used fluo-4 to monitor the intracellular Ca2+ dynamics in the neurons. After stimulating the non-conductive gel locally using a tungsten microelectrode, we observed sharp Ca2+ fluxes in the adjacent and downstream cells. This indicates that there was robust activation and communication between neurons within the hydrogel. Additionally, we measured the local field potential of the neuronal network within the gels and found activity characteristic of a communicating network. This study demonstrates the efficacy of porous 3D alginate hydrogels as scaffolds for developing neurons. Controlled growth of neurite processes using this scaffolding will be useful to better understand and direct cellular emergent behavior in differentiating and regenerating neurons.

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Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.10/A24

Topic: A.04. Axon and Dendrite Development

Title: Neurite outgrowth of dorsal root ganglion neurons via sensing environmental rigidity

Authors: *Y.-C. CHUANG[1,2], C.-C. CHEN[1,3];
[1]MM Program, TIGP, IBMS, Academia Sinica, Taipei, Taiwan; [2]Inst. of Biochem. and Mol. Biology, Natl. Yang-Ming Univ., Taipei, Taiwan; [3]Taiwan Mouse Clinic, Natl. Comprehensive Mouse Phenotyping and Drug Testing Center, Academia Sinica, Taipei, Taiwan

Abstract: Substrate stiffness is an important mechanical cue affecting neuronal development and regeneration. Neurons of dorsal root ganglion (DRG) have been indicated owning potential to sense different rigidity of environment since peripheral nervous system is supported by a variety of tissue. Few investigations, however, have been conducted on the mechanism how peripheral neurons sense environmental rigidity. In this study, we applied polydimethylsiloxane with various types of stiffness responding to different tissue elasticity as culture substrates, and established the relationship between the substrate rigidity and the outgrowth pattern of DRG neurons. The results, similar to the phenomena in spinal cord, showed that neurite developed enriched branches on soft material. Furthermore, we found advillin, as a sensory neuron-specific and actin binding protein, regulated neurite processing in N2a cells, affected the morphology of growth cone and promoted the neurite branching in cultured DRG neurons. Proteomic approach

indicated advillin interacted with non-muscle myosin IIa and IIb, which have been reported modulating dynamics of growth cones and rigidity sensing. The subcellular distribution in growth cones revealed the interaction between advillin and myosin IIa/b affected by substrate stiffness. These observations implied that interaction of advillin and myosin II contributed to neurite outgrowth via mechanotransduction. Our work discovered the importance of actin-regulating protein on neuronal navigation on substrates with different elasticity, and could benefit future studies on neural tissue engineering.

Disclosures: Y. Chuang: None. **C. Chen:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.11/A25

Topic: A.04. Axon and Dendrite Development

Support: NSF Grant 1054168

Title: Slit-roundabout signaling during post optic commissure formation in zebrafish forebrain development

Authors: *N. E. WREN[1], J. M. SCHNABL[2], B. EDENS[1], E. DESCHENE[1], J. PARK[1], A. ANTOINE[1], K. ALLIGOOD[1], M. HARDY[1], C.-B. CHEN[3], M. J. F. BARRESI[1];
[1]Smith Col., Northampton, MA; [2]Univ. of Massachusetts Amherst, Amherst, MA; [3]Dept. of Neurobio. and Anat., Univ. of Utah, Salt Lake City, UT

Abstract: During embryonic development, neurons send pioneering axons to make necessary connections, and establish the fundamental circuitry of the brain. With the help of environmental guidance cues, these axons navigate vast distances to reach and form synapses with target neurons. To connect both hemispheres of the brain, pathfinding axons lay the groundwork for the formation of commissures, tight bundles of axons that cross the midline of the brain. A substrate of astroglia cells, characterized as an ‘astroglial bridge’ in the zebrafish forebrain, provides adhesive support and directional guidance for midline crossing axons of the Post-optic commissure (POC). At the midline, axons are guided in part by Slits, extracellular signaling molecules, and their receptor, Roundabout, which is expressed at the growth cone of extending axons. In bilateral animals *slit* is known to act as an axon repellant, and prevent axons from wandering into regions of the brain they are not intended occupy. Preliminary data in the Barresi laboratory has suggested a previously uncharacterized role for Slit1a to positively mediate

growth-promoting interactions between axons and astroglia. To investigate this role, the Barresi laboratory uses a *gli2DR* (*you-too, yot*) zebrafish mutant that results in the loss of POC formation due to the expansion and upregulation of *slit2* and *slit3* expression and coincident downregulation of *slit1a*. In order to rescue POC commissure formation in *yot*, a transgenic heatshock promoter line [*tg(hsp70:slit1a-mCherry); yot*] was generated to return *slit1a* to the system. Transplants of gastrula stage *tg(hsp70:slit1a-mCherry* into the telencephalon of wild-type gastrula stage embryos were also used to assess the effect of local *slit1a* misexpression on POC axons. Additionally, hypothetical Roundabout mutants (*robo1 and robo4)* have been generated to investigate their roles as potential receptors for *slit1a*. Using Geographic Information System (GIS) spatial analysis tools, the Barresi laboratory further aims to more objectively define biological phenomena, including the characterization POC phenotypes. GIS tools are being used to calculate mean fluorescence values to determine spatial patterns, establish statistical significance of fluorescence clustering, calculate average axon movement to assess wandering or defasciculation of the POC, and measure sizes of anatomical structures in the forebrain. These tools will help further evaluate effects of Slit-Robo signaling on POC formation.

Disclosures: **N.E. Wren:** None. **J.M. Schnabl:** None. **B. Edens:** None. **E. Deschene:** None. **J. Park:** None. **A. Antoine:** None. **K. Alligood:** None. **M. Hardy:** None. **C. Chen:** None. **M.J.F. Barresi:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.12/A26

Topic: A.04. Axon and Dendrite Development

Support: Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT, No. 1070377)

Millennium Nucleus RC120003

Ring Initiative ACT1109

FONDECYT postdoctoral grant, 3130527

Title: Schwann cell-derived exosomes enhance neurite extension of adult sensory neurons via modulation of axonal guidance pathways

Authors: *F. PICOU[1], B. DIAZ[1], D. DIAZ DOMINGUEZ[2], P. MANQUE[2], F. A. COURT[1]; [1]Pontifical Univ. Catolica De Chile, Santiago, Chile; [2]Ctr. de Genómica y Bioinformática, Univ. Mayor, Santiago, Chile

Abstract: Nervous system function relies on the coordinated action of neurons and glial cells. Phenomena like synaptic activity, conduction of action potentials or axonal growth, to name a few, are fine tuned by glial cells. The mechanistic basis of glial cell-neuron communication is only partially understood. Recently, horizontal transfer between cells of proteins, lipids and nucleic acids through exosomes (40-100 nm vesicles) has been described. Our lab found that exosomes secreted by Schwann Cells (SC, peripheral nervous system glial cells) are able to increase neurite extension of embryonic dorsal root ganglion (DRG) sensory neurons *in vitro* and *in vivo*. We used purified primary SC culture and adult DRG neurons, SC-exosome function was studied using biochemistry and microscopy. Exosome RNA content was identified by next generation sequencing and qPCR. Bioinformatic analysis (IPA analysis) was used for network reconstruction and miRNA target gene prediction. SC-derived exosomes increase neurite extension in adult DRGs after 24 and 48 hours *in vitro*. Next generation sequencing of neuronal samples highlights an important modulation of axon guidance pathway in exosome-treated neurons. We also describe that SC-derived exosomes partially mimics a sciatic nerve conditioning lesion, a well-described paradigm to enhance neurite extension of adult DRG *in vitro*. Moreover, only few mRNA and miRNA carried by SC exosomes are able to efficiently modulate this axonal guidance pathways. Our results suggest that SC-derived exosomes tpartially recapitulates conditioning lesion effect on neuronal cells *in vitro* at the morphological and transcriptomic level. The underlying mechanism by which SC exosomes contribute to neurite outgrowth is a promising strategy to enhance axonal regeneration in both central and peripheral nervous systems.

Disclosures: F. Picou: None. **B. Diaz:** None. **D. Diaz Dominguez:** None. **P. Manque:** None. **F.A. Court:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.13/A27

Topic: A.04. Axon and Dendrite Development

Support: AHA NCRP Scientist Development Grant 11SDG5280029

Mt. Sinai Scholar Program

Title: Nerve Growth Factor regulates TRPV2 via ERK signaling to enhance neurite outgrowth

Authors: *V. Y. MOISEENKOVA-BELL, M. R. COHEN, W. M. JOHNSON, J. M. PILAT, J. KISELAR, A. DEFRANCESCO-LISOWITZ, R. ZIGMOND;
Dept. of Pharmacol., Case Western Reserve Univ., Cleveland, OH

Abstract: Neurite outgrowth is key to formation of functional circuits during neuronal development. Neurotrophins including nerve growth factor (NGF) increase neurite outgrowth in part by altering the function and expression of Ca2+-permeable cation channels. Here we report that TRPV2 is an endosomal Ca2+-permeable transient receptor potential vanilloid (TRPV) channel that augments neurite outgrowth mediated by NGF, clearly indicating that the channel functions in intracellular membranes. TRPV2 protein levels increased after NGF treatment, suggesting that TRPV2 may be involved in NGF-induced neurite outgrowth. Overexpression of a Ca2+-impermeable TRPV2 mutant or TRPV2 knockdown significantly reduced neurite outgrowth in PC12 cells after NGF treatment. Pharmacological assays revealed MAPK signaling contributes to upregulation of TRPV2 in developing sensory neurons and PC12 cells. Furthermore, we show that TRPV2 acts as an ERK substrate in the processes of developing neurons and identify the structural elements necessary for the phosphorylation of TRPV2 by ERK, leading to enhanced TRPV2-mediated Ca2+ signals and neurite outgrowth. Based on these findings, we propose that TRPV2 is a component of the neuronal signaling endosomes and is directly modulated by ERK, altering Ca2+ signaling within neurites to enhance neurite outgrowth.

Disclosures: V.Y. Moiseenkova-Bell: None. **M.R. Cohen:** None. **W.M. Johnson:** None. **J.M. Pilat:** None. **J. Kiselar:** None. **A. DeFrancesco-Lisowitz:** None. **R. Zigmond:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.14/A28

Topic: A.04. Axon and Dendrite Development

Support: Medical Research Foundation of Oregon

NSF GRFP DGE1448072

Title: Dystroglycan regulates visual circuit development

Authors: *K. WRIGHT, R. CLEMENTS;
Vollum Inst., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: The interpretation of cues in the extracellular environment by cell surface receptors is critical for proper neural circuit development. Dystroglycan is a transmembrane receptor that provides a link from the extracellular matrix (ECM) to the actin cytoskeleton. The ability of dystroglycan to bind to ligand requires its extensive glycosylation, and mutations that affect dystroglycan glycosylation lead to the development of congenital muscular dystrophy. Human patients with severe forms of dystroglycanopathy, including Walker Warburg Syndrome (WWS) and Muscle-Eye-Brain disease (MEB), present with severe neurodevelopmental defects, including type II lissencephaly, hindbrain malformations and retinal dysplasias. Using ISPDL79*/L79* mutant mice that lack glycosylated dystroglycan as a model system to study the mechanistic basis that underlie the neuropathological defects in WWS/MEB, we have identified a critical role for dystroglycan in regulating neuronal migration and axon guidance during the development of visual circuits. We find that in the absence of functional dystroglycan, the inner limiting membrane of the retina breaks down and neurons over-migrate, resulting in the formation of extensive neuronal ectopias in the vitreal space. The axons of developing retinal ganglion cells (RGCs) in ISPDL79*/L79* mutant mice exhibit guidance defects within the retina and at the optic chiasm, resulting in abnormal innervation of retinorecipient areas in the brain. We are using conditional deletion of dystroglycan to identify the specific cell types in which it functions, and to determine the role of dystroglycan in postnatal stages of visual circuit development, including laminar stratification within the retina, refinement of axonal targeting to retinorecipient areas of the brain and formation of functional synapses. Ultimately, these studies will define how dystroglycan regulates neuronal migration and axon guidance during neural circuit development, and provide mechanistic insight into the neuropathological defects observed in WWS/MEB.

Disclosures: **K. Wright:** None. **R. Clements:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.15/A29

Topic: A.04. Axon and Dendrite Development

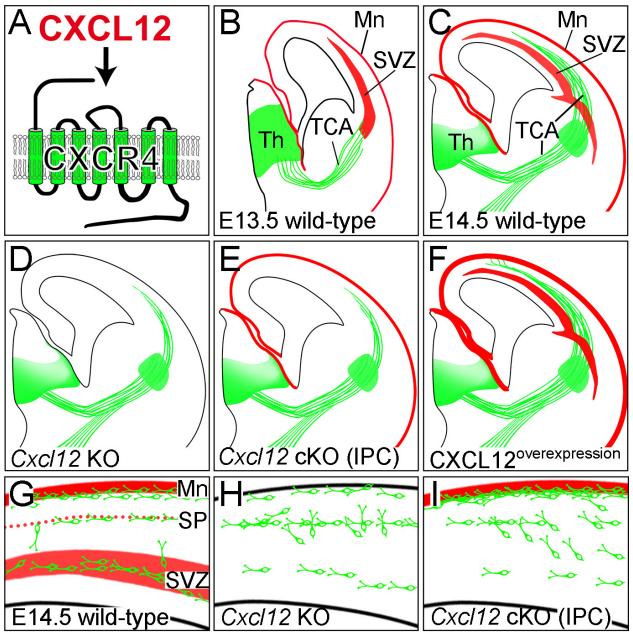
Support: STU 295/7-1

AR732/1-1

Title: Intermediate progenitors facilitate intracortical progression of thalamocortical afferents and interneurons through cxcl12 chemokine signaling

Authors: *P. ABE[1], Z. MOLNAR[2], Y.-S. TZENG[3], D.-M. LAI[4], S. J. ARNOLD[5], R. STUMM[1]; [1]Inst. of Pharmacol. and Toxicology, Jena Univ. Hospital, Friedrich Schiller Unive, Jena, Germany; [2]Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom; [3]Grad. Inst. of Oncology, Natl. Taiwan Univ., Taipei, Taiwan; [4]Dept. of Surgery, Natl. Taiwan Univ. Hosp. and Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan; [5]Renal Div., Univ. Hosp. Freiburg, Freiburg, Germany

Abstract: Glutamatergic principal neurons, GABAergic interneurons and thalamocortical afferents (TCAs) are essential elements of the cerebrocortical network. Principal neurons originate locally from radial glia and intermediate progenitors (IPCs) whereas interneurons and TCAs are of extrinsic origin. Little is known how the assembly of these elements is coordinated. C-X-C motif chemokine 12 (CXCL12), which is known to guide axons outside the neural tube and interneurons in the cortex, is expressed in the meninges and IPCs. We dissected the influence of IPC-derived CXCL12 on TCAs and interneurons by showing that Cxcl12 ablation in IPCs, leaving meningeal Cxcl12 intact, attenuates intracortical TCA growth and disrupts tangential interneuron migration in the subventricular zone. Mechanistically, our data suggest that intracortical TCA growth is regulated by crosstalk between growth-promoting CXCL12 and repellent cortical plate-derived Slit1. Thus, a CXCL12 signal from IPCs links cortical neurogenesis to the progression of TCAs and interneurons spatially and temporally.



Abbreviations: Mn, meninges; MZ, marginal zone; SP, subplate; SVZ, subventricular zone; TCA, thalamocortical afferents; Th, thalamus

Disclosures: P. Abe: None. **Z. Molnar:** None. **Y. Tzeng:** None. **D. Lai:** None. **S.J. Arnold:** None. **R. Stumm:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.16/A30

Topic: A.04. Axon and Dendrite Development

Support: NIH R56 NS041564

NIH R21 NS088477

NIH T32 GM007507

Title: The role of growth cone invadosomes in chemotrophic axon guidance within 3D collagen gels

Authors: *C. A. SHORT, S. M. O'TOOLE, M. SANTIAGO-MEDINA, T. M. GOMEZ; Univ. of Wisconsin, Madison, WI

Abstract: Invadosomes are specialized F-actin rich protrusions from the basal surface of cells that function to adhere to and degrade the extracellular matrix to allow cells to invade new tissues. Invadosomes have largely been studied in cancer and immune cells, but recently have been identified in migrating neural crest cells in zebrafish and in pathfinding growth cones of developing Xenopus laevis spinal neurons (Santiago, et al. Development). Similar to non-neuronal cells, our lab found that the formation of invadosomes in neuronal growth cones requires Tsk5 activity, however, the extracellular cues and intracellular signaling molecules that induce the formation of these protrusions in neural cells are unknown. However, our previous work largely examined invadosomes in 2D cell culture. Here, we employ confocal and structured illumination microscopy (SIM) of developing motoneurons (MN) in 3D collagen gel to investigate the roles of candidate morphogens for invadosome formation and function. We hypothesize that growth factors, such as BDNF and SDF-1, released from peripheral tissues during development, promote invadosome formation necessary MN guidance into the periphery. Preliminary data suggest that spinal neurons axons are guided up a gradient of BDNF in 3D collagen gels. Invadosome-dependent penetration of collagen gel and guidance toward local growth factor sources in 3D is being examined. Future studies will examine the molecular basis of invadosome formation and function *in vivo*.

Disclosures: C.A. Short: None. **S.M. O'Toole:** None. **M. Santiago-Medina:** None. **T.M. Gomez:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.17/A31

Topic: A.04. Axon and Dendrite Development

Support: Wings for Life Spinal Cord Research Foundation, WFL-CA-008/14

Title: Molecular mechanisms of MAG and myelin-induced Smad2 phosphorylation

Authors: J. L. C. CADIEUX, S. SELAMAT, *S. S. HANNILA;
Human Anat. and Cell Sci., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Inhibitory proteins in myelin such as myelin-associated glycoprotein (MAG), Nogo-A, and oligodendrocyte myelin glycoprotein pose a significant barrier to axonal regeneration in the central nervous system (CNS) after injury. Previous studies have shown that all three proteins bind to Nogo receptor 1 (NgR1) and paired immunoglobulin receptor B (PirB) with high affinity, and MAG also binds to low-density lipoprotein receptor-related protein-1 (LRP1). In addition to these receptors, we now show that myelin-associated inhibitors can also activate the transforming growth factor β (TGFβ) receptor and Smad2/3 signaling pathway. For P6 rat cerebellar granule neurons (CGN), P1 cortical neurons, and P1 hippocampal neurons, treatment with 20 µg/ml MAG-Fc, Nogo-AP, or CNS myelin for 30 minutes strongly induced phosphorylation of Smad2. In our recent work, we have also shown that myelin basic protein (MBP), one of the most abundant proteins in myelin, does not induce Smad2 phosphorylation, which indicates that this effect is specific to myelin-associated inhibitors. To elucidate the role of the TGFβ receptor in this process, P6 CGN were treated with the TGFβ receptor inhibitors SB431542 and SB505124 for 1 hour, followed by the addition of 20 µg/ml MAG-Fc for 30 minutes. In samples from neurons that received SB431542 or SB505124, MAG-induced Smad2 phosphorylation was completely blocked, which suggests that MAG activates the TGFβ receptor. We are now characterizing the onset and duration of Smad2 activation by myelin-associated inhibitors and investigating whether the TGFβ receptor is being transactivated by the known MAG receptors NgR1, PirB, and LRP1. Elucidating the pathways involved in mediating inhibition of axonal growth is the first step towards developing therapies for enhancing functional recovery after spinal cord injury, and ultimately, we plan to test whether TGFβ receptor inhibitors are capable of overcoming inhibition by myelin and promoting axonal regeneration *in vivo*.

Disclosures: J.L.C. Cadieux: None. **S. Selamat:** None. **S.S. Hannila:** None.

Poster

029. Axon Growth and Guidance: Extrinsic Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 29.18/A32

Topic: A.04. Axon and Dendrite Development

Title: A quantitative study of the locally synthesized proteins in the axons of rat cortical neurons in culture

Authors: *H.-W. CHUNG;
Dept. of Life Sci., Instituate of Mol. Medicine, Natl. Tsing H, Taoyuan City, Taiwan

Abstract: Local protein synthesis as enhanced by environmental stimulations may play important roles in neuronal growth, axonal guidance and synaptic plasticity[1]. Recently, it has been found that both glutamate and brain-derived neurotrophic factor (BDNF) treatments enhance protein translation not only in cell bodies but also in axons disconnected from their cell bodies of rat cortical neurons in culture. Here, we have used the bioorthogonal noncanonical amino acid tagging (BONCAT) [2], which has been widely used in studying nascent proteins, to label the newly synthesized proteins in the axons and whole cells of cultured rat cortical neurons. By quantitative Western blotting, we have compared the newly synthesized proteins in cell bodies and axons of cultured rat cortical neurons. The results indicate that the set of proteins whose syntheses are enhanced by BDNF is nearly identical to that as enhanced by glutamate, either in whole neurons or in severed axons. However, the sets of proteins whose syntheses are enhanced by BDNF and glutamate in whole neurons are respectively different from those in severed axons. In addition, we have also calculated the proportions of newly synthesized proteins in the total amounts of proteins in the axon in the basal level and those upon stimulation with glutamate and BDNF. The results will be presented. The results will help understand the mechanism(s) of how local protein synthesis may contribute to axon's functions. Reference:1. Kang, H., Schuman, E. M., A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. Science 1996, 273, 1402-1406. 2. Kalil, K.Li, L.Hutchins, B. I. Signaling mechanisms in cortical axon growth, guidance, and branching. Front Neuroanat 2011, 5:62-75

Disclosures: H. Chung: None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.01/A33

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: MEXT KAKENHI 22115009

MEXT KAKENHI 15H01454

MEXT KAKENHI 15H04263

Grain-in-Aid for JSPS Fellows

Title: Long-term 2-photon imaging of thalamocortical connectivity refinement in the neonatal mouse barrel cortex

Authors: *S. NAKAZAWA[1,2], H. MIZUNO[1,2], T. IWASATO[1,2];
[1]Natl. Inst. of Genet., Mishima, Shizuoka, Japan; [2]Dept. of Genet., SOKENDAI (The Grad. Univ. for Advanced Studies), Mishima, Shizuoka, Japan

Abstract: Sensory information through the thalamus flows to the primary sensory cortex layer 4 (L4), and thalamocortical (TC) connectivity is refined by thalamic inputs during early postnatal period. In the mouse somatosensory cortex (barrel cortex) L4, there is an array of "barrels" that correspond to the arrangement of whiskers on the face. In barrel cortex L4, there are 2 types of excitatory neurons: spiny stellate neurons (barrel cells) and star pyramid neurons, which are distinguished by the absence and presence of apical dendrites, respectively. Barrel cells are located around the barrel edge, and their dendrites are asymmetrically oriented toward the barrel center, where TC axon termini are clustered. These unique morphological features of barrel cells allow single barrel cells to receive inputs from single whiskers. We are interested in how these barrel cell morphologies that are critical bases of precise TC connectivity are established during early postnatal period. To address this question, *in vivo* 2-photon time-lapse imaging should be a powerful approach. For the purpose, we labeled L4 excitatory neurons sparsely and brightly using the *in utero* electroporation-based "Supernova"-RFP (Mizuno et al., Neuron 2014), and visualized barrel center using the TCA-GFP Tg mouse (Mizuno et al., 2014). We then analyzed TC refinement by 18 hour-long *in vivo* imaging starting from postnatal day 5 (P5), and revealed that barrel cells reinforced their dendritic orientation toward the barrel center by moving dendritic branches dynamically (Mizuno et al., 2014). Our histological analyses demonstrated that most morphological features of barrel cells are apparent at P6 (and P5) but not at P3. To reveal dynamics and mechanisms of the initial phase of TC connectivity refinement, we here performed 3-day long *in vivo* imaging of L4 excitatory neurons starting at P3. In this meeting, we

will show how morphological differences between barrel cells and star pyramid neurons arise and how barrel cells acquire characteristic dendritic orientation bias during neonatal period.

Disclosures: **S. Nakazawa:** None. **H. Mizuno:** None. **T. Iwasato:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.02/A34

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: Swiss National Science Foundation

Title: Activity-dependent controls over L4 glutamatergic interneuron identity

Authors: ***I. VITALI**[1], L. TELLEY[1], S. BARISELLI[2], N. HURNI[1], A. DAYER[1], C. BELLONE[2], D. JABAUDON[1];
[1]Fundamental Neurosci., Univ. of Geneva, Geneve, Switzerland; [2]Fundamental Neurosci., Univ. of Lausanne, Lausanne, Switzerland

Abstract: Neuronal circuits are thought to arise from a coordinated interplay between cell-intrinsic transcriptional programs, which form the backbone of the differentiation process, and superimposed input/activity-dependent programs, which act to refine neuronal identity in a circuit-specific manner. The whisker-to-barrel cortex pathway is ideally suited to study these hierarchical interactions in single neuron types during circuit assembly. We have recently shown that modality-specific thalamocortical input instructs the identity of their postsynaptic partner in layer 4 (Pouchelon et al., Nature, 2014), but whether L4 neuron activity is required for differentiation and circuit assembly is unknown. Here, using cell-type specific manipulations of L4 glutamatergic interneurons during development, we identify an activity-dependent control over the laminar, molecular, morphological and circuit identity of this cell type. Our experiments reveal an early and unexpected role for intrinsic activity on the functional differentiation of specific cortical neuron cell types, which suggests that cell-intrinsic and input/activity-dependent processes are tightly intermingled and regulated during early corticogenesis.

Disclosures: **I. Vitali:** None. **L. Telley:** None. **S. Bariselli:** None. **N. Hurni:** None. **A. Dayer:** None. **C. Bellone:** None. **D. Jabaudon:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.03/A35

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant R01EY013613

Simons Foundation SFAR1205440

P30OHD018655

NiH F31 Grant 5F31NS083437-03

Title: Cortical feedback regulates feedforward retinogeniculate remodeling during a thalamic critical period

Authors: *A. THOMPSON[1,2], L. MIN[3], M. FAGIOLINI[4], C. CHEN[4];
[1]F.M. Kirby Neurobio. Dept., [2]BBS Program, [3]Neurobio., Harvard Univ., Boston, MA; [4]F.M. Kirby Neurobio. Dept., Harvard University, Children's Hosp. Boston, Boston, MA

Abstract: The development of sensory pathways has traditionally been thought to occur in a feedforward manner, such that local microcircuits refine and stabilize in isolation before directing the wiring of each subsequent layer. In the visual system, retinal circuits were thought to mature first and direct refinement in the visual thalamus (the lateral geniculate nucleus), after which cortical circuits would mature and incorporate visual experience during well-defined critical periods. Here we show that feedback from cortex to thalamus directly regulates the refinement of the retinal projection to thalamus during a discrete window in development. Disrupting cortical activity during this time frame leads to rewiring of the subcortical circuit characterized by an increase in the number of retinal ganglion cells innervating a given thalamic relay neuron. Both decreasing and increasing corticothalamic activity elicits this response, indicating that corticothalamic firing patterns may actively instruct the rewiring of the primary afferent input to thalamus. Our results demonstrate that primary sensory pathways develop through the concurrent and interdependent remodeling of subcortical and cortical circuits in response to sensory experience, rather than through a simple feedforward process. Moreover, our findings highlight an unexpected function for the corticothalamic projection.

Disclosures: **A. Thompson:** None. **L. Min:** None. **M. Fagiolini:** None. **C. Chen:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.04/A36

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: Ellison Medical Foundation

W.M. Keck Foundation

Title: Physical exercise-induced neurotransmitter re-specification in the adult mouse brain

Authors: *H. LI, K. JACKSON, N. SPITZER;
Neurobio. Section, Div. of Biol. Sciences, Kavli Inst. for Brain, UCSD, LA Jolla, CA

Abstract: The identity of the transmitters expressed by neurons has been thought to be fixed and immutable. However changes in electrical activity can reconfigure the transmitters and corresponding receptors that neurons express, both in the developing and adult brain (Borodinsky et al., 2004; Borodinsky & Spitzer, 2007; Dulcis & Spitzer, 2008; Demarque & Spitzer, 2010; Dulcis et al., 2013). Functionally, neurotransmitter switching in the adult rat hypothalamus has been shown to regulate behavior (Dulcis et al., 2013). The existence and behavioral consequences of neurotransmitter switching in other brain regions remain largely unknown. We thus began to search for activity-dependent neurotransmitter switching in motor circuitry where the mechanism underlying plasticity remains obscure. Our strategy is to compare the number of neurons expressing cFos in motor nuclei of mice with free access to running wheels to the number in nuclei of sedentary control mice. We found that mice exercised by running for one week showed a robust 5-fold increase in the number of cFos+ cells in the midbrain pedunculopontine nucleus (PPN) accompanied by a 33% decrease in the number of choline acetyltransferase-immunoreactive (ChAT+) neurons. Stereological counts showed that the decrease in number of ChAT+ neurons in the PPN occurred exclusively in the caudal region that projects to locomotor centers in the brain stem, to STN, VTA, and to the thalamus. There was no change in number of ChAT+ neurons in the rostral PPN that projects to other nuclei. The number of ChAT+ neurons in the adjacent laterodorsal tegmental nucleus was also unaffected. TUNEL staining revealed the absence of apoptosis. Moreover, nitric oxide synthase (NOS) is a marker for ChAT+ neurons and there was no change in the number of NOS+ neurons in the caudal PPN. The number of ChAT+ neurons was restored one week after mice had stopped running. These data are consistent with transmitter re-specification. To determine the consequence of ChAT loss in the caudal PPN, we performed behavioral analysis and found that runners progressively increased the duration of running episodes and acquired better motor control during training on running wheels. They also performed better than controls on the

rotorod motor learning and coordination test; no significant difference was seen in the locomotor activity test, the open field test, or metabolic analysis. To test for causal relationships, we are determining whether preventing ChAT loss can suppress the promotion of motor learning by running. This study is expected to be pertinent to understanding the mechanism underlying motor learning as well as neurological disorders of the motor system.

Disclosures: H. Li: None. **K. Jackson:** None. **N. Spitzer:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.05/A37

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Title: Early BDNF signaling can substitute for visual experience in maintenance of receptive field refinement in dark-reared adults

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Abstract: Critical periods allow external information to shape sensory circuits during postnatal development, limiting plasticity in adulthood. Sensory deprivation interferes with this process, often leading to retention of the plastic state. Surprisingly, visual experience is not required for the refinement of retinocollicular or visual cortical receptive fields (RFs) (Balmer and Pallas, 2015). Instead, early light exposure protects against the deterioration of high acuity, refined RFs in adulthood (Carrasco et al., 2005). The failure to maintain refined RFs in dark-reared adults results primarily from a loss of inhibitory drive and thus a reduction in lateral inhibition (Carrasco et al., 2011). We sought to identify the experience-driven molecular trigger that stabilizes GABAergic synapses and maintains refined RF size. The neurotrophic factor BDNF and its receptor TrkB have been implicated as a link between visual experience, maturation of inhibition, and ocular dominance plasticity in visual cortex. We tested whether BDNF-TrkB signaling during the critical period also provides a mechanism for maintenance of RF refinement. If so, then TrkB activation during a critical period in dark-reared animals should substitute for light exposure and thus maintain refined RFs in adults. Pharmacological manipulation of TrkB signaling in an *in vivo* rodent model was used to generate an increase in TrkB phosphorylation during postnatal development. Syrian hamsters were dark-reared from birth to adulthood (>P90) and given daily IP injections of the TrkB agonist 7’8 Dihydroxyflavone or vehicle during the

critical period for RF refinement in the superior colliculus (SC) (P33-P37). Single unit *in vivo* extracellular recordings in superficial SC were used to compare adult RF sizes between treatment groups. We found that animals treated with the TrkB agonist (n = 26 units, mean RF diameter = 12.6 ± 0.81 deg), but not animals treated with vehicle alone (n = 16, mean = 17.9 ± 1.24 deg), maintained refined RFs into adulthood ($p<0.001$, t-test), suggesting that activation of the TrkB signaling pathway mimics visual experience. Thus, early, visually-driven activity appears to promote maintenance of RF refinement in SC via up-regulation of BDNF and subsequent TrkB phosphorylation. Our findings suggest that the SC and visual cortex share a common mechanism for experience-driven plasticity across distinct RF properties, despite differences in timing of their critical periods. The importance of TrkB signaling in modulating different critical periods across brain regions may facilitate investigation of pathological conditions in which maturation is governed by inhibitory plasticity.

Disclosures: D.B. Mudd: None. **T.S. Balmer:** None. **S.L. Pallas:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.06/A38

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH P01 NS062686-04

Title: Effects of Chronic Hypoxia on Excitatory/Inhibitory balance in postnatal development

Authors: *D. XENOS[1], M. KAMCEVA[1], N. SALMASO[1,4], F. VACCARINO[1,2,3];
[1]Child Study Ctr., [2]Dept. of Neurobio., [3]Kavli Inst. for Neurosci., Yale Univ., New Haven, CT;
[4]Dept. of Neurosci., Carleton Univ., Ottawa, ON, Canada

Abstract: Very low birth weight (<1500 grams) prematurely-born infants, frequently experience periods of hypoxia due to immaturity of their respiratory system, that can severely affect brain development and result in lasting cognitive and behavioral impairments. In our study we used a mouse model of chronic postnatal hypoxia to examine the impact of hypoxic rearing (9.5-10.5% O2) from postnatal day 3 (P3) to P11. We previously showed that hypoxia transiently decreases the number of excitatory neurons and impairs the maturation of specific GABAergic interneuron populations together with a significant reduction of the mice cortical volume. Here, we investigated whether hypoxia may have also affected the maturation of synaptic connectivity in the neocortex of young adult mice. We found that there is a substantial decrease in presynaptic

inhibitory (vGAT) terminal boutons impinging onto pyramidal cell bodies in the layer 5 and layer 1 of the motor cortex in young adult hypoxic-reared mice. In the same layers, we detected a significant decrease in the number of presynaptic excitatory (vGlut1) terminal boutons impinging onto the soma of PV+ interneurons hypoxic-reared mice as compared to littermate controls. We identified similar patterns in the somatosensory cortex, where we also identified a decrease in thalamocortical excitatory (vGlut2) terminals. These findings were not reflected by a generalized loss of synaptic proteins, measured by western blot analysis. Moreover, using Golgi-cox impregnation, we detected a short-term decrease of both basilar and apical dendritic spines in cortical pyramidal neurons in the hypoxic motor cortex at P15 followed by normalization at P35, but persistent decreased dendritic branching and an immature morphology of the spines. All these results were paralleled by alterations in proteins downstream the mTOR pathway in the hypoxic cortex at young adulthood. Our data show that hypoxia disrupts the anatomical organization of cortical synapses with the molecular mechanisms behind this to being currently examined. To highlight these anatomical changes we are performing whole cell reconstruction of specific neuronal populations in hypoxia labeled with reporter AAV vectors, as well as electrophysiological *in vivo* recordings to understand the functional impact of these abnormalities.

Disclosures: **D. Xenos:** None. **M. Kamceva:** None. **N. Salmaso:** None. **F. Vaccarino:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.07/A39

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant NS015918

NIH Grant NS057690

Title: Investigating intracellular signaling events involved in neurotransmitter-receptor matching

Authors: *D. R. HAMMOND-WEINBERGER[1], N. C. SPITZER[1,2];
[1]Neurobio. Section, Div. of Biol. Sci., UCSD, La Jolla, CA; [2]Kavli Inst. for Brain and Mind, La Jolla, CA

Abstract: Matching the identity and location of postsynaptic neurotransmitter receptors to the transmitters released from presynaptic nerve terminals is a substantial challenge. The number of

neurotransmitters is large and the number of synapses most neurons receive is much larger. To begin to analyze the mechanism of neurotransmitter-receptor matching, we have focused on the developing *Xenopus* neuromuscular junction, where motor neurons normally express the neurotransmitter acetylcholine (ACh) and muscle cells express cognate ACh receptors. Changes in electrical activity of these immature motor neurons during a critical period drive a homeostatic shift to non-cholinergic neurotransmitters and the retention of matching receptors from the initially diverse receptor pool. Reduced activity results in a switch from ACh to combinations of cholinergic and glutamatergic phenotypes, with the retention and upregulation of both ionotropic ACh and glutamate receptors in the postsynaptic cell (Borodinsky & Spitzer, 2007). The mechanism of receptor matching remains elusive. Cultured *Xenopus* muscle cells are sensitive to exogenous glutamate, as assayed with the fluorescent Ca^{2+} indicator Fluo-4AM, and sensitivity increases when myocytes are grown in the presence of glutamatergic neurons or exogenous glutamate. This increase in glutamate sensitivity is prevented by growing cells in the presence of pharmacological ionotropic glutamate receptor inhibitors. Thus, although genetic factors are important for initial transmitter and receptor expression, the presence of glutamate appears to be both necessary and sufficient to drive enhanced glutamate sensitivity in myocytes. Use of specific agonists and antagonists demonstrates that metabotropic receptors do not mediate the increased sensitivity to glutamate. Application of pharmacological antagonists suggests that enhanced sensitivity to glutamate depends on activation of mitogen-activated protein kinases (MAPKs) but not protein kinase A or protein kinase C. We are currently conducting Western blot analysis to determine the phosphorylation status of JNK and p38 MAPKs when cells are exposed to exogenous glutamate. We are also evaluating the contributions of specific downstream signaling cascades via targeted gene knockdown with the intent to test the *in vivo* role of transmitter receptor activation in generating postsynaptic receptor clusters (Yamanaka et al., 2013). Understanding the mechanisms of neurotransmitter-receptor matching in the nervous system could form the basis for devising therapies aimed at promoting functional recovery to damaged circuits.

Disclosures: D.R. Hammond-Weinberger: None. **N.C. Spitzer:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.08/A40

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NSERC

AIHS

Title: The effects of post-pubertal aging on the juvenile-induced changes in the development of the prefrontal cortex

Authors: *B. T. HIMMLER, S. M. PELLIS, B. KOLB;
Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Juvenile social interactions have been shown to influence the dendritic complexity of neurons in the prefrontal cortex. Juvenile social play has been shown to induce pruning of the cells in the medial prefrontal cortex (mPFC), whereas interacting with multiple partners, whether those interactions involve play or not, increases the complexity of cells in the orbital frontal cortex (OFC). Previous studies suggest that the changes in these different areas of the prefrontal cortex are differentially stable during adulthood. In the present study, rats were reared in groups of either four (quads) or two (pairs) and the brains of the rats from each rearing condition were then harvested at 60 days (i.e., shortly after sexual maturity) and 100 days (i.e., fully adult). The rats housed with multiple partners had more complex neurons of the OFC at 60 days and this complexity declined to a comparable level to that of pair housed rats by 100 days. The play-induced pruning of the mPFC remained similar at both ages. These findings suggest that the changes in the prefrontal cortex induced by social experiences in the juvenile period differ in how long they are maintained in adulthood. The differences in the functions regulated by the OFC and the mPFC may account for these differences on the effects of age on neural complexity.

Disclosures: B.T. Himmler: None. **S.M. Pellis:** None. **B. Kolb:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.09/A41

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant R01-NS082761-01

AES-Post-Doctoral Fellowship

Title: Postnatal expression of Arx in GABAergic interneurons is critical for proper network function in the mouse hippocampus

Authors: *D. J. JOSEPH, A. J. MCCOY, R. RISBUD, E. D. MARSH;
Pediatrics Neurol., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Transcription factors (TFs) establish molecular codes that orchestrate early stages of interneuron development. Some TFs remain expressed in mature interneurons, suggesting a divergent, yet unknown, function. We previously showed that postnatal conditional ablation of one TF, the Aristaless Related homeobox gene (Arx), in parvalbumin (PV) positive interneurons resulted in learning deficits, alterations in intrinsic and synaptic properties, and electrographic seizures in some animals. These results suggest that Arx loss in all mature interneurons may have a more profound effect on network function. Thus, we studied the effect of postnatal ablation of Arx in all interneurons on the excitability of hippocampal networks. To temporally control Arx expression, we crossed a floxed Arx mouse (Arxfl/fl) with a tamoxifen-inducible CreER- mouse. Cre-mediated recombination induced by tamoxifen (Tam) injection in P35-40 male mice resulted in ablation of Arx in interneurons. Tam-induced (Arx-/y;CreER) and sham injected (Arx+/y;CreER) mice were sacrificed 10 days after the injections and processed for immunohistochemistry to assess the efficiency of Cre-mediated recombination. To determine the consequence on network activity, intracranial EEG and extracellular field recordings were performed ~21 days after the injections. Mice were monitored by video EEG continuously for 5 days. Field potentials were recorded at the hippocampal CA1 region in response to Schaffer collaterals (SC-CA1) stimulation. As an index of synaptic transmission, we examined the input-output (I/O) coupling of field excitatory postsynaptic potentials (fEPSPs). To examine the effect of Arx loss on synaptic plasticity, we recorded fEPSPs and measured the probability of release and long term plasticity (LTP). Preliminary results demonstrated that Tam injections resulted in nearly complete ablation of Arx in cortical and subcortical brain regions. Background EEG showed some irregular spikes, but no seizure was observed after Tam injections. Ablation of Arx postnatally resulted in a leftward shift of the I/O curve, suggesting a reduction in synaptic transmission. Paired-pulse stimulation of SC-CA1 was not affected by ablation of Arx, suggesting no change in release probability. However, both HFS and theta burst stimuli failed to induce robust LTP in the Arx-/y; CreER mice, indicating that loss of Arx in interneurons impaired LTP. Our preliminary study suggests that postnatal loss of Arx may alter network function via impairments of synaptic transmission and LTP. Therefore, postnatal expression of Arx may regulate the maturation of functional properties of inhibitory interneurons.

Disclosures: **D.J. Joseph:** None. **A.J. McCoy:** None. **R. Risbud:** None. **E.D. Marsh:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.10/A42

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: Epigenomics Flagship Project EPIGEN MIUR-CNR

Title: Mir 132 regulates the development of binocular matching of orientation preference in the mouse visual cortex

Authors: R. MAZZIOTTI[1], P. TOGNINI[2], J. TOLA[2], G. CHELINI[1], D. NAPOLI[3], D. SILINGARDI[2], *N. BERARDI[2], G. DELLA SALA[1], E. PUTIGNANO[2], L. BARONCELLI[2], T. PIZZORUSSO[1];
[1]NEUROFARBA, Univ. of Florence, Florence, Italy; [2]Inst. Neurosci. del CNR, Pisa, Italy; [3]Scuola Normale Superiore, Pisa, Italy

Abstract: MicroRNA-132/212 (miR-132/212) is an experience and cAMP response element-binding protein (CREB) dependent MicroRNA (miRNA) that acts in the central nervous system and in peripheral tissue regulating important biological processes, such as circadian clock, spine maturation and neural inflammation. Recently miR- 132/212 has been involved in Ocular Dominance (OD) plasticity during the critical period in mouse visual cortex. We have studied OD plasticity and binocular matching in MicroRNA-132/212 Knockout (miR-132/212 KO) mice, where the genomic locus of miR-132/212 is completely deleted. To examine the role of miR-132/212 in visual cortical function, we analyzed Local Field Potentials (LFP) responses to pattern Visual Evoked Potential (VEP) and measured single units activity to drifting sine gratings, in Wild Type (WT) and miR-132/212 KO mice. We found that the preferred orientations of individual cortical cells are mismatched through the two eyes at a significant higher level in animals that lack of miR-132/212 and monocularly deprived mice, respect to aged-matched WT subjects. Furthermore, as seen before, three days of Monocular Deprivation (MD) were not sufficient to induce OD shift during the critical period in miR-132/212 KO mice, assessed using pattern VEP responses. These results suggest a possible role of miR-132/212 in trigger adaptive rewiring of neuronal circuits following visually driven patterned activity.

Disclosures: R. Mazziotti: None. **P. Tognini:** None. **J. Tola:** None. **G. Chelini:** None. **D. Napoli:** None. **D. Silingardi:** None. **N. Berardi:** None. **G. Della Sala:** None. **E. Putignano:** None. **L. Baroncelli:** None. **T. Pizzorusso:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.11/A43

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant MH051234

NIH Grant P50 MH103204

Title: Developmental trajectories of parvalbumin-positive chandelier and basket cells in prefrontal cortex circuits

Authors: *T. MIYAMAE, O. KRIMER, D. A. LEWIS, G. GONZALEZ-BURGOS;
Dept. of Psychiatry, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Parvalbumin (PV)-positive neurons regulate maturation of cortical circuit function. Moreover, PV neuron alterations, one of the best replicated findings in schizophrenia research, are thought to arise during development. Despite the relevance of PV neuron maturation for cortical development, the maturation trajectories of the PV neuron subtypes, chandelier and basket cells (ChCs and BCs), are poorly understood. We therefore assessed the maturation of ChCs and BCs during development of the prefrontal cortex (PFC), a cortical region that plays a crucial role in the development of cognitive function and its alterations in schizophrenia. We recorded from GFP+ PV neurons with soma localized near the layer 1/layer 2 (L1/L2) border in acute slices of the PFC of G42 mice (P12-P60). At P12, many GFP+ cells had BC-like axonal morphology, but some had markedly different axonal arbors in which we detected vertical cartridges of boutons, albeit in very small numbers, consistent with immature ChCs. At P14-15, the number of cartridges per ChC axon increased, and overall axon morphology was comparable to that of mature ChCs. As in other cortical regions, in PFC ChCs had asymmetric dendrites preferentially projecting to L1, while the L1/L2 dendrite distribution was more symmetric in BCs. These different L1/L2 dendrite distributions were also observed in cells classified as ChCs or BCs in slices from P12 mice. In both ChCs and BCs, firing properties changed gradually into a fast spiking (FS) phenotype during PFC development, as revealed by measurements of 10 electrophysiological parameters in 35 ChCs and 54 BCs (P12-P60). Seven parameters changed progressively with age, and the rate of change was well fit by single exponential curves. Interestingly, the time constants of the exponential curve fits indicated a slower development of FS properties in ChCs than BCs. Furthermore, the time to reach 95% of plateau (mature) value for the 7 curve fits was substantially longer for ChCs than BCs (29±2.9 vs 18±0.6 days), and the plateau values for most parameters differed between ChCs and BCs. Preliminary analysis of AMPA receptor-mediated EPSCs (P12-P30) suggests a different maturation of excitatory synaptic inputs in ChCs versus BCs. Our data suggest different trajectories of functional development in ChCs and BCs, consistent with the possibility that they play significantly different roles in regulating the activity and maturation of other circuit elements during PFC development. Their distinct developmental trajectories may determine a different sensitivity of

ChCs and BCs to the effects of environmental/genetic risk factors thought to disturb PV neuron development in schizophrenia.

Disclosures: **T. Miyamae:** None. **O. Krimer:** 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.; Pfizer. F. Consulting Fees (e.g., advisory boards); Autifony Therapeutics, Bristol-Myers Squibb, Concert Pharmaceuticals, Sunovion Pharmaceuticals. **G. Gonzalez-Burgos:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.12/A44

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Title: Expression of Npas4 mRNA in telencephalic areas of adult and postnatal mouse brain

Authors: *U. H. WINZER-SERHAN, J. C. DAMBORSKY;
Dept Neurosci & Exp. Therap., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: The transcription factor neuronal PAS domain-containing protein 4 (Npas4) is an inducible immediate early gene which regulates the formation of inhibitory synapses. Npas4 could have a significant regulatory role during the development of cortical circuits and in shaping the excitatory/inhibitory balance. However, little is known about the expression of Npas4 mRNA under normal conditions, and no study has systematically evaluated the expression of Npas4 during postnatal development, when GABAergic synapses are formed. To address these questions, adult and postnatal mouse brain sections were processed for isotopic *in situ* hybridization using a Npas4 specific cRNA probe. The results show that adult expression of Npas4 is found in the telencephalon with very restricted or no expression in the diencephalon or and mesencephalon. In most telencephalic areas, including the anterior olfactory nucleus (AON), piriform cortex, neocortex, hippocampus, dorsal caudate putamen (CPu), septum and basolateral amygdala nucleus (BLA), Npas4 expression was found in scattered cells which exhibited strong hybridization signal. In the postnatal mouse brain, transcripts for Npas4 mRNA were detected in AON, CPu and piriform cortex at P5. At P8, additional Npas4 hybridization was found in CA1 and CA3 stratum pyramidale of dorsal and ventral hippocampus, and in primary motor cortex. By P13, robust mRNA expression was located in layers IV and VI of all sensory cortices and in cingulate cortex. The spatial distribution of postnatal mRNA expression was similar to that in

adults, except for the CPu, where Npas4 became gradually restricted to the most dorsal part. In conclusion, the spatial distribution of Npas4 expression was mostly restricted to telencephalic areas, and the developmental expression of Npas4 mRNA correlated with the maturation of excitatory synapses, when activity-driven formation of inhibitory synapses becomes more important. Understanding the temporal and spatial expression of Npas4 will serve as a foundation to explore the potential roles of Npas4 during development, and for the characterization of stimuli that could activate Npas4 expression.

Disclosures: U.H. Winzer-Serhan: None. **J.C. Damborsky:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.13/A45

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Title: Short term potentiation induced by theta burst stimulation in CA1 pyramidal cells depends on activation of protein-G

Authors: *M. OUARDOUZ;
NRAC, Gatineau, QC, Canada

Abstract: As previously reported blocking G-protein by $GDP_{\beta s}$ induces an increase of EPSCs amplitude (Ouardouz, 2014). The increase in EPSCs amplitude by $GDP_{\beta s}$ may interfere with the expression of long term potentiation (LTP) of excitatory synapses in CA1 pyramidal cells. We used theta burst protocol (5 trains of 4 pulses at 100 Hz at 200 ms interval, 3 times at 30s interval) for LTP induction in CA1 pyramidal cells from P9-12 rat pups. In control condition theta burst protocol induces an increase of EPSCs amplitude which decay with time corresponding to short-term potentiation (EPSCs amplitude increases by 39.8 $\pm$ 4.5 %, n = 5, p = 0.0000004) flowed by a persistent increase that last for the duration of the recordings (30 minutes, EPSCs amplitude increases by 26.4 $\pm$ 4.4 %, n = 5, p = 0.00005). When $GDP_{\beta s}$ is included in the recording solution, theta burst stimulation induces a progressive EPSCs amplitude increase. The short term potentiation observed in control is not present. After 30 minutes recording the EPSCs amplitude was increased by 54.5 $\pm$ 4.6 % after theta burst stimulation compared to 25.7 $\pm$ 3.7 % without theta burst stimulation both in the presence of GDP_{β}. Paired pulse facilitation due to increase transmitter release at the presynaptic terminal was also explored. The increase of the amplitude of the second response compared to the first one is not different between control and in the presence $GDP_{\beta s}$ (% EPSCs amplitude increase in control

50.3 ± 9.8 %, n = 6 and in the presence of $GDP_{\beta s}$ 50.7 ± 7.6 %, n = 5, p = 0.98). However, after 30 minutes induction of LTP by theta stimulation, paired pulse facilitation is more pronounced in control group (90.7 ± 11.4 % after burst compared to 50.7 ± 7.6 % before Theta burst, P = 0.04 paired t-test) compared to cells recorded in the presence of $GDP_{\beta s}$ (45.5 ± 3.8 % after theta burst compared to 50.7 ± 7.6 % before Theta burst, P = 0.58). Those results show that short term potentiation was affected by intracellular application of $GDP_{\beta s}$ suggesting a role of G-protein activation in the expression of short-term potentiation. In addition, $GDP_{\beta s}$ block the increase paired pulse facilitation induced by theta burst stimulation in CA1 hippocampus suggesting the involvement of diffusing messenger from the post- to the pre-synaptic side.

Disclosures: M. Ouardouz: None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.14/A46

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: MEXT KAKENHI No. 23115102

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JSPS KAKENHI No. 25640035

JSPS KAKENHI No. 15K14350

Title: Activity-dependent dynamics of CREB in cortical neurons: a single-molecule imaging study

Authors: H. KITAGAWA, *N. SUGO, N. YAMAMOTO;
Osaka Univ., Suita, Osaka, Japan

Abstract: Activity-dependent transcription is essential for neuronal circuit formation and synaptic plasticity. An intriguing question is how neuronal activity affects dynamic interactions between sequence-specific transcription factors and their target sites. Here we investigated activity dependence of DNA binding and dissociation of cAMP-response element binding protein (CREB) in living cortical neurons. To visualize CREB at single molecule level, HaloTag-CREB was transfected into dissociated mouse cortical neurons, and was labeled with a single tetramethylrhodamine (TMR)-conjugated HaloTag ligand. Then, the individual TMR-CREB

molecules were observed in living cell nuclei by highly inclined and laminated optical sheet microscopy. At 5 DIV, when spontaneous firing activity is low, a significant fraction of TMR-CREB spots resided at restricted positions for several seconds (dissociation rate constant: 0.54 s^{-1}), but mutant CREB which lacks binding to its target sequence cAMP-response element (CRE) scarcely stayed at fixed locations for such a long period. To test the possibility that the residence time depends on neuronal activity, an optogenetic method with Channelrhodopsin-2 was applied to cultured cortical neurons. Although the photostimulation efficiently induced nuclear translocation of CREB-regulated transcriptional coactivator 1 (CRTC1), the residence time distribution of CREB was not changed before and after the photostimulation. However, we found that TMR-CREB spots with long residence times (> 1s) repeatedly emerged at highly restricted nuclear locations (0.8 × 0.8 μm) after the photostimultation. Quantitative analysis revealed that the number of the accumulation sites per optical section significantly increased in response to the optogenetic stimulation. Taken together, these results suggest that neuronal activity promotes CREB-dependent transcription by accelerating the frequency of CREB binding to particular CRE sites without altering the temporal binding property.

Disclosures: H. Kitagawa: None. **N. Sugo:** None. **N. Yamamoto:** None.

Poster

030. Activity-Dependent Neural Circuit Development and Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 30.15/A47

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: 104AC-B7, Aim for the Top University Plan from the Ministry of Education, Taiwan

Title: Hypoxia-ischemia induces gephyrin misfolding via calcineurin-dependent dephosphorylation of growth-associated protein 43 in developing cortical neurons

Authors: C.-Y. WANG[1,2], H.-C. LIN[2], P.-C. HSU[1], Y.-M. KUO[2,4], C.-H. WENG[2], C.-Y. LEE[2], W.-H. CHIEN[2], C.-H. LIN[5], *Y.-H. LEE[3,1,2];
[1]Brain Res. Ctr., [2]Physiol., [3]Natl. Yang-Ming Univ., Taipei, Taiwan; [4]Anesthesiol., Taipei Veterans Gen. Hosp., Taipei, Taiwan; [5]Kang-Ning Junior Col. of Med. Care and Mgmt., Taipei, Taiwan

Abstract: Growth-associated protein 43 (GAP43) is a protein kinase C-dependent phosphoprotein known to promote activity-dependent presynaptic plasticity and axon growth. In this study, we revealed for the first time that phosphorylation of GAP43 at PKC phosphorylation

site Ser41 is pivotal for postsynaptic gephyrin clustering in developing cortical neurons. We found that GAP43 regulates the aggregation of gephyrin, a pivotal protein for clustering postsynaptic GABAA receptors (GABAARs), in developing cortical neurons. Pharmacological blockade of PKC and neuronal activity induced both GAP43-gephyrin association and gephyrin misfolding in the cytosol and aggresomes, suggesting the importance of PKC-dependent regulation of GABAergic synapses. Furthermore, we found that PKC phosphorylation-resistant GAP43S41A, but not PKC phosphorylation-mimicking GAP43S41D, interacted with gephyrin to trigger gephyrin misfolding and its sequestration into aggresomes. Interestingly, gephyrin clustering was greatly reduced by GAP43S41D, but not GAP43S41A. Surface GABAAR expression, which is highly dependent on gephyrin in developing neurons, were also reduced by GAP43S41D overexpression under physiological condition. In contrast, GAP43S41D can attenuate gephyrin misfolding under transient oxygen-glucose deprivation (tOGD) that mimics hypoxia-ischemic insult in neonatal brains. Calcineurin-mediated GAP43 dephosphorylation that accompanied tOGD also led to GAP43-gephyrin association and gephyrin misfolding/aggregation. Together, this study highlights the importance of GAP43 phosphorylation in the development of inhibitory synapses against hypoxic insult via a novel regulation of gephyrin aggregation.

Disclosures: **C. Wang:** None. **H. Lin:** None. **P. Hsu:** None. **Y. Kuo:** None. **C. Weng:** None. **C. Lee:** None. **W. Chien:** None. **C. Lin:** None. **Y. Lee:** None.

Poster

031. Transplantation and Regeneration

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 31.01/A48

Topic: A.07. Transplantation and Regeneration

Support: China Postdoctoral Science Foundation 20100481475

Title: Transplantation of pro-oligodendroblasts, preconditioned by lps-stimulated microglia, promotes recovery after acute contusive spinal cord injury

Authors: ***X. LIN**[1], T. ZHAO[2], M. WALKER[3], A. DING[1], M. JIANG[1], J. CAO[1], S. LIN[1], X.-M. XU[3], S. LIU[1];
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Abstract: Spinal cord injury (SCI) is a significant clinical challenge and, to date, no effective treatment is available. Oligodendrocyte progenitor cell (OPC) transplantation has been a promising strategy for SCI repair; however, the poor post-transplantation survival and deficiency in differentiation into myelinating oligodendrocytes (OLs) are two major challenges, which limit their use as donor cells. Here we report the generation of a relatively more mature OL lineage population, i.e. pro-oligodendroblast (proOL), than OPC, for transplantation after SCI. We found that proOLs responded to lipopolysaccharide (LPS)-stimulated microglia conditioned medium (L+MCM) by preserving toll-like receptor 4 (TLR4) expression, improving cell viability, and enhancing myelinating capability as compared to other OL lineage cells exposed to either LPS-stimulated or non-stimulated microglia conditioned medium (L-MCM). When stimulated proOLs were intrathecally delivered through a lumbar puncture after a T10 thoracic contusive SCI, they promoted behavior recovery assessed by Basso- Beattie-Bresnahan (BBB) locomotor rating scale, stride length, and slips on the grid tests. Histologically, transplantation of stimulated proOLs caused a considerable increase in axon growth and myelination, and less accumulation of macrophages at the lesion site when compared with the vehicle control or OPC transplantation group. Comparing with direct transplantation of OPCs, inducing these cells to a more mature proOL stage and stimulating them with L+MCM may be more advantageous since the proOLs may have already initiated the differentiation process towards OLs prior to transplantation. Thus, transplantation of proOLs, preconditioned by LPS-stimulated microglia conditioned medium, may offer a better therapeutic potential than OPCs for recovery after acute SCI.

Disclosures: **X. Lin:** None. **T. Zhao:** None. **M. Walker:** None. **A. Ding:** None. **M. Jiang:** None. **J. Cao:** None. **S. Lin:** None. **X. Xu:** None. **S. Liu:** None.

Poster

031. Transplantation and Regeneration

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 31.02/A49

Topic: A.07. Transplantation and Regeneration

Support: Emerging Technology Funds from the State of Texas to A.K.S.

VA Merit Award to A.K.S.

Title: hiPSC-derived NSC grafting intervention early after hippocampus injury preserves memory and mood function and reduces the occurrence of seizures

Authors: *B. HATTIANGADY[1,2,3], A. BATES[1,2,3], S. SHIN[4], B. SHUAI[1,2,3], X. RAO[1,2,3], M. C. VEMURI[4], A. K. SHETTY[1,2,3];
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Abstract: The hippocampus, a region of the brain vital for memory and mood function, exhibits its highest level of plasticity in response to injury. While some of these post-injury changes are reparative in nature, the other changes contribute to the development of aberrant synaptic reorganization and epileptogenesis. Hence, even a partial injury to the hippocampus may result in the occurrence of chronic temporal lobe epilepsy, typified by memory and mood impairments and spontaneous recurrent seizures (SRS). Here, we ascertained the efficacy of human induced pluripotent stem cell (hiPSC)-derived neural stem cell (NSC) grafts for restraining the evolution of unilateral hippocampal injury into conditions such as memory and mood dysfunction and chronic epilepsy. We first induced partial injury to the right hippocampus of young adult F344 rats through an administration of kainic acid (KA; 0.25 μg in 1 μl of saline) into the right lateral ventricle. Seven days after injury, a cohort of rats received grafts of hiPSC-derived NSCs expanded in xeno-free culture conditions (Life Technologies) into the injured hippocampus (~100,000 live cells to each of 3 sites) and another cohort received sham-grafting surgery. Animals in both groups received daily injections of an immunosuppressant drug cyclosporine until euthanasia. Animals that received sham-grafting surgery displayed spatial and recognition memory impairments in water maze and object recognition memory tests and increased depressive-like behavior in a forced swim test, when examined 3-4 months after injury. In contrast, animals that received hiPSC-derived NSC grafts displayed ability for formation of spatial and recognition memories and normal mood function at similar post-injury time-points. Measurement of SRS using chronic EEG recordings suggested that NSC grafting after injury decreased both frequency and duration of EEG Seizures by ~60%. Characterization of the grafted hippocampus using human nuclear antigen (HNA) revealed excellent survival and migration of graft-derived cells. Dual immunofluorescence analyses for HNA and neural cell markers revealed differentiation of a larger fraction (>80%) of graft-derived cells into NeuN+ mature neurons and smaller fractions of graft-derived cells into GABA+ inhibitory interneurons, S-100ß+ mature astrocytes and NG2+ oligodendrocyte progenitors. Analyses with Ki-67 revealed proliferative activity in only a minority of graft-derived cells (<1%). These results underscore that, early hiPSC-derived NSC grafting intervention into the injured hippocampus is effective for preserving memory and mood function and diminishing the occurrence of seizures.

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Poster

031. Transplantation and Regeneration

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Topic: A.07. Transplantation and Regeneration

Support: MRC grant mr/j004553/1

NIHR Biomedical Research Centre for Ophthalmology

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Wellcome Trust/MRC 4 year Neuroscience Studentship

UCL Institute of Ophthalmology

UCL Grand Challenge Studentship

Title: Effective transplantation of cones and cone-like cells into the diseased retina is dependent upon the recipient environment

Authors: *P. V. WALDRON[1,2], G. GRIMALDI[3,1], A. B. GRACA[2,1], F. DI MARCO[3,1], C. HIPPERT[2,1], J. CLAUDIO RIBEIRO[2,1], S. J. I. BLACKFORD[2,1], N. AGHAIZU[2,1], Y. DURAN[2,1], A. J. SMITH[2,1], J. W. B. BAINBRIDGE[2,1], J. SOWDEN[3,1], R. R. ALI[2,1], R. A. PEARSON[2,1];
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Abstract: Replacement by photoreceptor (PR) transplantation represents a possible strategy for restoring vision in retinal degenerative diseases. We, and others, have shown that post-mitotic rod precursors can be transplanted into diseased adult eyes, where they functionally integrate within the recipient retina. Human vision is primarily dependent on cone PRs and the transplantation of cones is likely to be the most useful application of PR replacement. We previously provided proof of concept for cone transplantation. However, our data suggested that the adult retinal environment, while receptive to new cones, negatively impacts the number of integrating cone cells, favouring rod differentiation and integration. Transcription factors *Nrl* and *Nr2e3* act as co-activators of rod genes while suppressing cone gene expression. In *Nrl* and *Nr2e3* deficient retinae, rod development is impaired and a majority of cells develop as cones. We sought to use the plasticity of PR precursors by transplanting precursor cells genetically committed to a cone-like fate. Methods: 200 000 cone-like PR precursors were isolated from Nrl$^{-/-}$NrlGFP or Nr2e3^{rd7}CrxGFP mice and transplanted into recipient mice of several genotypes,

representing wild-type, cone-only (*Nrl*$^{-/-}$), cone functionless (*cpfl5*), cone depleted (*cpfl1*) and slowly degenerating (*Prph2*$^{rds/rds}$) retina. The R91W;Nrl$^{-/-}$ mouse was used as a non-degenerating cone-only phenotype. True cone precursors were also isolated from Chrnb4.eGFP mice and transplanted into wild-type and Nrl$^{-/-}$ retinae. Results: Cone and cone-like PR precursors integrated into all recipients and produced unambiguous examples of PR morphology. When transplanted into recipients with rod-rich environments (*wild-type, cpfl1, cpfl5*), the majority of integrated cells of either origin resembled rod PRs, although some resembled cones. In contrast, cells integrated into rod-depleted, cone-enriched environments (*Nrl*$^{-/-}$, R91W;Nrl$^{-/-}$, *Prph2*$^{rds/rds}$) more typically resembled cones. Significantly more cells integrated into *Prph2*$^{rds/rds}$ and *Nrl*$^{-/-}$ retinae than other recipients. Conclusions: The recipient environment affects the outcome of cone transplantation. Specifically, the rod/cone balance of the recipient retina affects the fate of integrated cells and their morphological features while factors within the recipient environment, including OLM integrity and degeneration, influence the efficiency of transplantation. Containing only cones, the Nrl$^{-/-}$ retina has similarities with the fovea of the human retina. Successful transplantation into this environment represents an important advance in the development of this technique.

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Poster

031. Transplantation and Regeneration

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 31.04/A51

Topic: A.07. Transplantation and Regeneration

Support: Rush Translational Sciences Consortium

Title: Dopamine neurons derived from human iPSCs retain midbrain phenotype in animal models of Parkinson's disease

Authors: *D. R. WAKEMAN[1], B. M. HILLER[1], D. J. MARMION[1], C. W. MCMAHON[2], G. T. CORBETT[1], J. MA[2], J. H. KORDOWER[1];
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Abstract: Human induced pluripotent stem cells (iPSCs) are a promising source of autologous midbrain dopamine neurons for transplantation in Parkinson's disease (PD). Efficient differentiation of iPSCs into functional midbrain lineage dopamine neurons (iPSC-mDA) utilizing floor-plate induction has been shown to ameliorate functional deficits in animal models of PD. Cryopreservation of post-mitotic iPSC-mDA neurons represents a significant advancement towards translation of iPSC-mDA cell therapy for PD. Here, we demonstrate that cryopreserved iPSC-mDA neurons can be reliably thawed, allowing for rapid access to large numbers of highly pure neurons. Furthermore, we examined the engraftment potential of iPSC-mDA neurons after transplantation into both the rodent and nonhuman primate brain. Human iPSC-mDA neurons derived by episomal reprogramming were cryopreserved in master cell banks. Following thaw, iPSC-mDA neurons retained high viability for >1-month *in vitro* with gene and protein expression profiles consistent with the midbrain dopaminergic lineage. Electrophysiological recordings demonstrated both spontaneous and evoked action potentials, as well as functional Na^+ and K^+ ion channels with characteristic dose-dependent responses to pharmacological inhibition. Biochemical analysis of iPSC-mDA neurons indicated dopamine secretion. To determine *in vivo* survival, cryopreserved iPSC-mDA neurons were thawed and prepared for transplantation without additional subculturing. Cyclosporine immunosuppressed Sprague-Dawley rats received bilateral stereotactic injections of iPSC-mDA neurons ($4.5x10^5$ cells/hemisphere) into the striatum or substantia nigra and sacrificed 2- or 6-weeks post-transplantation. MPTP-treated cynomolgus macaques received three injections to the post-commissural putamen bilaterally ($3.75x10^6$ cells/hemisphere) and were sacrificed 4-weeks or 3-months post-transplantation. Immunohistochemical analysis demonstrated robust graft survival and maintenance of the midbrain dopaminergic phenotype with extensive fiber innervation into the host. We found no evidence of cell proliferation, indicating safety in our initial studies. Long-term functional studies are underway to ascertain whether cryopreserved iPSC-mDA neurons will provide functional benefit in the 6-OHDA-lesioned rat and MPTP-lesioned nonhuman primate models of PD. These results demonstrate excellent graft survival, maintenance of the midbrain dopaminergic phenotype, lack of neural overgrowth in parkinsonian rats and monkeys, as well as indicate considerable promise for the development of pluripotent cell-based therapies in PD.

Disclosures: **D.R. Wakeman:** 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.; Cellular Dynamics International, Inc.. **B.M. Hiller:** None. **D.J. Marmion:** None. **C.W. McMahon:** None. **G.T. Corbett:** None. **J. Ma:** None. **J.H. Kordower:** None.

Poster

031. Transplantation and Regeneration

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 31.05/A52

Topic: A.07. Transplantation and Regeneration

Support: CT Stem Cell Initiative

Title: Embryonic stem cell-derived neural progenitors:interactions with the neurovasculature

Authors: *C. LASSITER, J. GAL, S. BECKER, L. GRABEL;
Biol., Wesleyan Univ., Middletown, CT

Abstract: Before we can safely use embryonic stem cell (ESC)-based cell replacement, we must characterize the behavior of these cells following transplant. We use embryonic stem cell-derived neural progenitors (ESNPs) to study the relationship between blood vessels and neural progenitors. The vasculature plays major roles in neural progenitor cell regulation, both during development and adulthood. Transplants of mouse ESNPs to the dentate gyrus region of the hippocampus are richly vascularized, and surprisingly, the ESNPs appear to migrate great distances from the original site of injection. Migrating ESNPs are found in close proximity to endogenous blood vessels, outside of the transplant area. Our data suggest that blood vessels and their associated astrocytes may provide a source of the chemokine CXCL12, which promotes cell migration in the brain. To test this model, we use organotypic hippocampal slice culture and find that human ESNPs are closely associated with blood vessels and this association increases over time. To directly study the interaction between ESNPs and endothelial cells and identify a molecular mechanism, we co-culture human ESNPs with mouse brain endothelial cells (BECs). We find the adhesive interaction between these two cell types involve an integrin-mediated binding. We examined the interaction of ESNPs with BECs *in vitro* and observe a dramatic morphological rearrangement leading to clusters of ESNPs between endothelial cells. Human ESNPs migrate towards BECs, in a Boyden chamber assay, likely due to factors secreted from the endothelial cells. These data suggest that the vasculature can promote ESNP migration and raise the concern that in a clinical application transplanted ESNPs will migrate away from the original transplant, and be disruptive at a distant site. On the other hand, understanding the signaling that directs neuronal migration may lead to more successful targeting of cells to areas of neurodegenerative damage.

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Poster

031. Transplantation and Regeneration

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 31.06/A53

Topic: A.07. Transplantation and Regeneration

Support: Chinese National Natural Science Foundation (No. 81330 028; U1301223)

Title: Construction of a spinal cord-like tissue *in vitro* to repair spinal cord injury

Authors: *B. Q. LAI, JR[1], Y. S. ZENG[2];
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Abstract: Recovery of damaged neural network after spinal cord injury is an unsettled scientific issue. Since the modern tissue engineering technology offer many powerful new tools for constructing engineered organoids *in vitro*. We herein attempt to take advantage of genetically modified neural stem cells (NSCs) and collagen sponge scaffolds, to generate a functional spinal cord-like tissue to reconstruct lesioned neural network of spinal cord through transplantation. The tissue is mimiced as real spinal cord containing white matter and grey matter. Our results showed, in white matter, many ciliary neurotrophic factor (CNTF) overexpressing oligodendrocytes forming thin myelin sheaths. In addition, NSCs transfected with either neurotrophin-3 (NT-3) or its high affinity receptor tyrosine kinase receptor type 3 (TrkC) genes tended to differentiate toward neurons which established synaptic connections between them in grey matter. When the white matter and grey matter tissues were assembled, we observed that axons from grey matter were attracted to white matter and enveloped by multilayered lamellae myelin. Furthermore, NSC-derived neurons in grey matter were revealed forming mature synaptic structures. Synapses of these neurons were found to be capable of releasing synaptic vesicles and to be able to record spontaneous post-synaptic currents. Our data suggest that the assembly of white matter and grey matter is expected to develop a functional spinal cord-like tissue and to offer insight into neural network reconstruction therapy after spinal cord injury.

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Poster

031. Transplantation and Regeneration

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Program#/Poster#: 31.07/A54

Topic: A.07. Transplantation and Regeneration

Support: NIH Area Grant #R15NS072879-01A1

Title: Enhanced GABAergic circuitry following Adeno-Associated Virus-Mediated Neuroligin2 overexpression in the hippocampus of adult mice

Authors: *M. A. VAN ZANDT[1], S. MAISEL[2], S. SHRESTHA[2], J. GUPTA[2], K. BUMSCH[2], F. HARRSCH[2], J. R. NAEGELE[2];
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Abstract: GABAergic interneuron dysfunction characterizes a number of neuropsychiatric disorders including autism and schizophrenia. Hippocampal GABAergic interneuron loss or dysfunction may also contribute to cognitive deficits or seizures in severe temporal lobe epilepsy (TLE). Stem cell therapy shows promise for treating interneuron dysfunction; GABAergic interneuron progenitor transplants in mice with TLE reversed cognitive deficits and ameliorated seizures after integrating synaptically into hippocampal circuitry (Henderson, Gupta et al; 2014, Hunt et al, 2013; Cunningham et al, 2015; Shetty et al, 2012). In the present study, we investigated whether Adeno-Associated Virus (AAV)-mediated overexpression of Neuroligin2 (NLGN2) in the hippocampus increased synaptic integration of endogenous or transplanted GABAergic interneurons. NLGN2 plays a pivotal role in forming and stabilizing GABAergic synapses during development, but little is known about its function in the adult brain. To overexpress NLGN2, we made stereotaxic injections into the hippocampus of AAV/DJ-CMV-mCherry-2a-mNLGN2 (titer: $6.4x10^{12}$ GC/mL; Vector BioLabs). Controls were injected with AAV/DJ-CMV-mCherry (Titer: 1.8×10^{13} GC/mL; Vector BioLabs). To observe the effects of NLGN2 overexpression on the structure of GABAergic synapses, we made stereotaxic injections of the virus into VGAT-ChR2(H134R)-eYFP adult male mice (Jackson Laboratories) and examined molecular components of GABAergic synapses 2-8 weeks later. The results showed that NLGN2 overexpression in principal cells in the adult hippocampus enlarges intracellular clusters of the post-synaptic scaffolding protein gephyrin, and they were apposed to GABAergic synaptic boutons. These findings suggest that AAV-mediated NLGN2 overexpression may be a powerful tool to manipulate the structure and location of GABAergic synapses in the adult brain. Current studies are testing the functional properties of these synapses using optogenetic approaches. Funding was provided by NIH Area #R15NS072879-01A1.

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Poster

031. Transplantation and Regeneration

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 31.08/A55

Topic: A.07. Transplantation and Regeneration

Title: Assessment of therapeutic efficacy and fate of GDNF expressing human induced pluripotent stem cell-derived neural precursor cells in cervical spinal cord injury

Authors: *M. KHAZAEI[1], N. NAGOSHI[1], H. NAKASHIMA[1], K. SATKUNENDRARAJAH[1], A. BADNER[1], A. MANN[1], M. SAYEG[1], M. G. FEHLINGS[1,2,3];
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Abstract: Spinal cord injury (SCI) is a common cause of disability and financial hardship that is currently without a cure. Cell therapies for treating SCI have shown great promise but are limited due to ethical concerns regarding cell source and challenges associated with the survival of graft cells. Human induced pluripotent stem cell derived neural precursor cells (hiPSC-NPCs) hold promise of being an autologous, patient-specific cellular therapy with less ethical concerns. The problems related to post transplantation survival of graft cells can be circumvented by GDNF, a neurotrophic factor that has been shown to promote survival of graft cells. However, delivery of GDNF *in vivo* is limited by its inability to cross the blood-brain barrier or penetrate gray matter, and by a relatively short half-life. To address this limitation we engineered the hiPSC-NPCs using piggyback vectors to express and secret GDNF. GDNF-expressing hiPSC-NPCs were transplanted into the spinal cords of a rodent model of cervical contusion SCI two weeks after injury and the involvement of these cells in neurobehavioural and neuroanatomical recovery was assessed. Our data suggest that the GDNF expressing cells significantly show higher survival and integration compared to control cell after transplantation and also protect host neurons resulting reduced cavity size, improved forelimb grip strength and better gait. In concordance, the amplitude of sensory evoked potential recordings was markedly higher in transplanted groups than in the medium-injected group.

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Poster

031. Transplantation and Regeneration

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 31.09/A56

Topic: A.07. Transplantation and Regeneration

Support: CIRM- RFA14-02

Title: Human neural stem cells restore cognitive impairment through trophic support in the Q140 knock-in mouse model of Huntington's disease

Authors: *A. RELAÑO GINÉS[1], C. ZHU[1], A. GALSTYAN[1], K. MOWRIS[1], M. S. LEVINE[2], L. M. THOMPSON[3], M.-F. CHESSELET[1];
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Abstract: Huntington's disease (HD) is an autosomal neurodegenerative disorder caused by an expanded CAG repeat in the huntingtin gene resulting in cognitive decline and motor dysfunction. No treatments currently exist for HD and there is an urgent need to develop new and effective therapies.The Q140 knock-in mouse model of HD displays early behavioral deficits in motor function and cognition as well as huntingtin aggregates, deficits in striatal transcripts and cortical brain-derived neurotrophic factor (BDNF).In this study, we assessed the therapeutic potential of a human neural stem cell line (ESI-017 NSC) (prepared by the UC. Davis GMP facility, Davis, CA) in the Q140 mouse model of HD. Littermates Q140KI and wild-type mice received vehicle instead of cells to be used as controls and all the mice in this study were immunocompromised by cyclosporine (2mg/Kg/day) that was administered by subcutaneous minipumps (Alzet#1004).After bilateral injection of 100,000 cells into the striatum, Q140 mice showed a transient improvement in motor function 3 months after transplantation, whereas notably, cognitive function was significantly improved as late as five months after cell transplantation. The cell transplants did not correct striatal transcripts anomalies measured 6 months after transplantation but levels of BDNF, as well as the levels of p-ERK, the downstream molecule of BDNF, were significantly increased and restored to wild-type levels in the striatum and cortex of the NSC treated mice.Our data suggest that the improved cognition may be mediated via a normalization of BDNF signaling and support the use of NSC for the treatment of HD.

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Poster

031. Transplantation and Regeneration

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Topic: A.07. Transplantation and Regeneration

Support: NYSTEM

Title: Survival and innervation of midbrain dopamine neurons derived from human embryonic stem cells transplanted in rodent and primate models of Parkinson's disease

Authors: *D. J. MARMION[1], B. M. HILLER[1], H. B. DODIYA[1], S. KRIKS[2], Z. XIE[3], D. J. SURMEIER[3], L. STUDER[2], J. H. KORDOWER[1], D. R. WAKEMAN[1];
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Abstract: The cardinal motor symptoms of Parkinson's disease (PD) result from the progressive neurodegeneration of dopamine (DA) neurons in the substantia nigra and subsequent loss of dopaminergic tone in the striatum. Transplantation of dopaminergic neurons derived from pluripotent stem cells represents a viable therapeutic option to ameliorate motor complications and replenish the diseased brain with a renewable source of DA. Simulating normal developmental signaling, human embryonic stem cells were differentiated into midbrain dopamine neurons (hESC-mDA) following floor-plate induction and midbrain dopaminergic patterning (Kriks et al., 2011). In an attempt to enrich for the midbrain lineage, magnetic activated cell sorting (MACS) was applied for cell surface marker CD142 at day-25 of differentiation. CD142 MACS and unsorted hESC-mDA neurons were transplanted bilaterally into the striatum of unlesioned Cyclosporin immunosuppressed Sprague-Dawley rats, 6-hydroxydopamine (6-OHDA)-lesioned Athymic RNU NUDE rats, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned macaque monkeys immunosuppressed with CellCept, Prograf, and prednisone. Animals were sacrificed along multiple time points, and the survival of grafted cells was assessed using human specific antigens. The maturation of transplanted neurons and maintenance of midbrain floor-plate lineage (FoxA2+/TH+) were analyzed up to 1-year post-transplantation. In rodents, grafted neurons had an immature, bipolar morphology at 2-weeks post-transplantation and developed into highly arborized mDA neurons by 6-months post-transplantation. Human specific neural cell adhesion molecule (huNCAM)+ fibers were seen coursing white matter tracts rostrally into the forebrain and caudally into the midbrain from striatal grafts as early as 2-weeks post-transplantation. By 6-months,

huNCAM+/TH+ fibers had extensively innervated the rodent substantia nigra. Characterization of grafted neurons revealed Girk-2+/TH+ and Calbindin+/TH+ cells, indicating that grafts contained both A9 and A10 lineage mDA neurons. Using Ki-67 as a marker for dividing cells, we saw no evidence for tumor formation up to 6-months post-transplantation. Long-term survival of CD142 sorted and unsorted hESC-mDA neurons were found up to 1-year post-transplantation in the MPTP-lesioned nonhuman primate model of PD. Grafted (FoxA2+/TH+) mDA neurons matured and showed extensive innervation into the host striatum. Functional studies are in progress to assess the therapeutic value and safety of hESC-mDA transplantation for PD.

Disclosures: D.J. Marmion: None. **B.M. Hiller:** None. **H.B. Dodiya:** None. **S. Kriks:** None. **Z. Xie:** None. **D.J. Surmeier:** None. **L. Studer:** None. **J.H. Kordower:** None. **D.R. Wakeman:** None.

Poster

031. Transplantation and Regeneration

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Topic: A.07. Transplantation and Regeneration

Support: NEI EY022589

NEI EY014801

Title: Integration of human embryonic stem cell-derived retinal ganglion cells after *in vivo* transplantation

Authors: *X. ZHANG[1], P. VENUGOPALAN[1,2], K. TENERELLI[3], C. SUN[1], J. GALVAO[1], K. MULLER[2], J. L. GOLDBERG[1];
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Abstract: Is it possible to repair retinas damaged by degenerative diseases like glaucoma, which injure and kill retinal ganglion cells (RGCs)? Cell therapies to replace RGCs will require a better understanding of both differentiation and integration. Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) may provide an unlimited source for cell transplantation therapy. We have developed a method of differentiating hESC/hiPSCs to RGCs using small molecules to inhibit BMP, Wnt and Notch signaling pathways. 30 days after

initiation of differentiation, RGC-like cells are generated from hESC/hiPSC culture that express RGC markers Brn3 and β-III-Tubulin and synapse marker synaptophysin. These cells respond to chemical stimulant KCl as determined by calcium imaging. Further, we have transplanted hESC-derived RGC like cells (hESC-RGCs) *in vivo* by intravitreal injection into young adult (1 month old) rat retinas, and studied the survival and integration of donor hESC-RGCs into host retinas. Our data suggest that donor hESC-RGCs integrate into the RGC layer as indicated by anti-human nuclei staining, with some transplanted cells co-labelled with RGC marker RBPMS and β-III-Tubulin. Second, we have transplanted primary mature RGCs *in vivo*. Donor RGCs from early postnatal transgenic GFP mice were transplanted into young adult rat eyes. Our data indicate that when transplanted intravitreally, the donor RGCs survive, extend neurites and functionally integrate into the host retina. Transplanted RGCs were observed to acquire the general morphology of endogenous RGCs with axons orienting towards the optic nerve head of the host retina as well as morphologic dendrites growing to the inner plexiform layer (IPL). GFP+ axons were also observed traversing the host optic nerves and optic tract to reach known RGC targets in the brain, e.g. the superior colliculus and lateral geniculate nucleus. Together these results indicate that RGC replacement therapy may be possible for diseases like glaucoma. Further experiments will compare the retinal transplant and integration of hESC/hiPSC-derived RGCs to primary RGCs and study their integration and function.

Disclosures: **X. Zhang:** None. **P. Venugopalan:** None. **K. Tenerelli:** None. **C. Sun:** None. **J. Galvao:** None. **K. Muller:** None. **J.L. Goldberg:** None.

Poster

031. Transplantation and Regeneration

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 31.12/A59

Topic: A.07. Transplantation and Regeneration

Support: DGAPA-PAPIIT: IB202112

DGAPA-PAPIIT: IN204612

CONACYT: 179927

Secretaría de Ciencia, Tecnología e Innovación del DF: PINV11-30

Title: Embryonic cell-derived cells as biosensors of regions that support neuronal differentiation within the adult brain

Authors: *O. COLLAZO-NAVARRETE[1], G. MAYA-ESPINOSA[2], D. MILLAN-ALDACO[3], M. PALOMARES-RIVERO[3], G. GUERRERO-FLORES[4], R. DRUCKER-COLIN[3], L. COVARRUBIAS-ROBLES[4], M. GUERRA-CRESPO[3];
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Abstract: The human motor functions can be permanently affected by the neuronal loss caused by a cerebral ischemic stroke or in association with neurodegenerative diseases such as Parkinson's disease (PD). Regenerative medicine supports the ability to restore the neuronal loss by using stem cells (SC). Pluripotent SC represent one of the most promising cell sources to achieve a successful treatment. However, until now there has not been systematically characterized the capacity of the adult brain to support neurogenesis of transplanted SC. It is unclear whether there are "silent" neurogenic niches or regions suitable for neuronal differentiation, in addition to the known areas of active neurogenesis. Considering the high neurogenic potential of embryoid bodies (EB) cells, derived from embryonic stem cells (ESCs), we used them as sensors to identify microenvironments in the adult rat brain with the capacity to support neuronal differentiation. We transplanted dissociated EB cells to conventional neurogenic and non-neurogenic regions and our results show a neuronal differentiation pattern of EB cells that was dependent on the host region. EB cell differentiation was initially patchy and progressed towards an even distribution along the graft by 15-30 days post-transplantation. The sequential expression of several differentiation markers along this time indicated: 1) EB cells transplanted into the striatum, a site conventionally considered non-neurogenic, showed a low level of neuronal differentiation while observing a significant number of astrocytes. 2) Remarkably, the EB cells shown that local ischemic stroke in the adult striatum promotes the formation of a favorable neuronal microenvironment, which increased the number of neuroblasts and neurons. 3) Unexpectedly, we found that the substantia nigra pars compacta (SNpc), a non-neurogenic region affected in PD, was highly permissive to the neurogenesis of EB cells. Therefore, our research validate the use of EB cells (SC uncommitted to the neural lineage) as biosensors of neurogenic environments in the adult brain. The relevance of this work is to point out by first time that the adult brain has favorable conditions for neuronal differentiation beyond the classic neurogenic sites, which not only helps to understand the limits of regeneration of mammalian brain, but also represents a fundamental step for the rational development of therapies with SC in neurodegenerative diseases.

Disclosures: O. Collazo-navarrete: None. **G. Maya-espinosa:** None. **D. Millan-aldaco:** None. **M. Palomares-rivero:** None. **G. Guerrero-Flores:** None. **R. Drucker-colin:** None. **L. Covarrubias-robles:** None. **M. Guerra-crespo:** None.

Poster

031. Transplantation and Regeneration

Location: Hall A

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Topic: A.07. Transplantation and Regeneration

Support: DOD Grant W91ZSQ2136N601 to R Keith Duncan

P30 DC005188

Title: A nanofiber guided approach for the integration of human neural precursors into the cochlea

Authors: *S. HACKELBERG[1], S. J. TUCK[2,3], A. RASTOGI[2], C. WHITE[2,3], L. LIU[1], D. M. PRIESKORN[1], R. MILLER[1], J. M. MILLER[1], J. M. COREY[2,3,4], R. K. DUNCAN[1]; [1]Otolaryngology, [2]Dept. of Biomed. Engin., Univ. of Michigan, Ann Arbor, MI; [3]Geriatrics Research, Education, and Clin. Ctr. (GRECC), VA Ann Arbor Healthcare Ctr. (VAAAHC), Ann Arbor, MI; [4]Dept. of Neurol., The Univ. of Michigan, Ann Arbor, MI

Abstract: The integrity of spiral ganglion neurons (SGN) of the inner ear can be compromised by age, genetics, infection, noise, blast exposure and skull fractures. This can lead to progressive auditory neuron loss with or without associated hair cell loss and affect the success of cochlear implants. Efforts to restore hearing thus need to provide strategies to secure the presence of functional SGNs. In mammals, SGNs lack the ability to spontaneously regenerate, making it necessary to promote replacement. A major hurdle in replacement of SGNs remains the proper integration of introduced neural precursor cells (NPC) with the host tissue. Nanofibrous scaffolds can potentially both guide the integration of NPCs and improve the ease of interaction with novel cochlear biohybrid implants. We have previously demonstrated that nanofibrous scaffolds promote the neurodifferentiation of mouse embryonic stem cells (Purcell et al., 2012). Here, we applied the use of polycaprolactone (PCL) nanofibrous scaffolds to human embryonic stem cells (hESCs). Briefly, hESCs were predifferentiated to derive NPCs and plated on plasma treated PCL fibers with average diameters below 1 μm. For optimal adhesion the cells preferred Matrigel or fibronectin coating over laminin and poly-l-lysine. Next, NPCs were allowed to terminally differentiate for 2-6 weeks in Neurobasal-based media. Consistent with previous studies on the biocompatibility of this material, we did not find signs of toxic effects interfering with cell viability. The progression of neural differentiation was examined at different time points in culture for lineage, maturation, and transmitter phenotype. Both aligned and unaligned nanofibers promoted the growth of human NPCs, which showed development of cellular extensions alongside as well as across single fibers. We confirmed the acquisition of a glutamatergic fate and synaptic structures. While we continue to study the impact of structure

and material on the differentiation and functional abilities of neurons grown on nanofibers, we have modified the nanofibrous scaffolds to study the integration of the neurons with the cochlear tissue. Methods have been developed for forming nanofiber conduits in bundled and rolled configurations along with surgical strategies for implantation into the internal auditory meatus.

Disclosures: **S. Hackelberg:** None. **S.J. Tuck:** None. **A. Rastogi:** None. **C. White:** None. **L. Liu:** None. **D.M. Prieskorn:** None. **R. Miller:** None. **J.M. Miller:** None. **J.M. Corey:** None. **R.K. Duncan:** None.

Poster

031. Transplantation and Regeneration

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CNPq

FAPERJ

CAPES

Ministério da Saúde

Title: Transplantation of mesenchymal stromal cells increases the survival and regeneration of retinal ganglion cells after optic nerve injury in adult rats

Authors: L. A. MESENTIER-LOURO[1,2], C. ZAVERUCHA-DO-VALLE[1,2], A. J. SILVA-JUNIOR[1,2], L. C. TEIXEIRA-PINHEIRO[1,2], G. NASCIMENTO-DOS-SANTOS[1,2], F. GUBERT[1,2], A. P. FIGUEIRÊDO[1,2], A. TORRES[1,2], B. D. PAREDES[1,2], C. TEIXEIRA[3], F. TOVAR-MOLL[3,4,5], M. F. SANTIAGO[1,2], *R. MENDEZ-OTERO[6,2];
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Abstract: We have investigated the therapeutic potential of mesenchymal stromal cells (MSC) transplanted after optic nerve crush (ONC), which causes retinal ganglion cell (RGC) death and optic nerve degeneration. Lister Hooded rats underwent unilateral ONC followed by an

intravitreal injection of 5 x 10^5 MSC (treated) or the vehicle (untreated). MSC were labeled with iron-oxide particles for cell tracking. Optomotor response was evaluated from 1 to 59 days after ONC. On the day before euthanasia, rats were kept for 24h in the dark and then exposed to light for 2h. The brains were analyzed for the expression of the immediate early gene NGFI-A in the SC. This gene is upregulated in neurons of the SC after activation of the NMDA receptor by RGC axons and it is downregulated in the absence of visual stimuli or connections from the retina to the SC. The retinas were immunostained for Tuj1 to estimate RGC number. Axons were anterogradely labeled with cholera toxin B (CTB). Tuj1-positive cell numbers in the central retina were increased from 88,88 ± 8,337 cells/mm^2 in the untreated group (n=9) to 260,4 ± 16,60 cells/mm^2 in the treated group (n=8, mean ± SEM, P<0.001), 28 days after injury. In addition, the number of CTB-positive axons 0.5 mm beyond the injury site was 2.7-fold increased in the treated group. MSC remained in the vitreous body for at least 18 weeks. Optomotor response was completely lost in the ipsilateral eye of treated and untreated groups, and it was not restored after 59 days. NGFI-A-positive cells in the contralateral SC were significantly increased from 0,1965 ± 0,02703 in the untreated group to 0,4059 ± 0,02377 in the treated group (% to ipsilateral SC, n=3, P<0,01). Cell counts 60 days after injury showed 67,98 ± 27,55 Tuj1-positive cells/mm^2 in the untreated group (n=3) and 104,1 ± 15,49 cells/mm^2 in the treated group (n=4). These preliminary results suggest that RGC degenerate overtime in both groups, but the number of cells is higher in the treated animals for at least 60 days after injury. Although this is not associated to the recovery of the optomotor response, NGFI-A expression was increased in the SC of treated animals, suggesting that axons regenerated and made synapses to their target cells in the brain. Further analysis of the visual response will be performed at 90 and 120 days after treatment.

Disclosures: **L.A. Mesentier-Louro:** None. **C. Zaverucha-do-Valle:** None. **A.J. Silva-Junior:** None. **L.C. Teixeira-Pinheiro:** None. **G. Nascimento-dos-Santos:** None. **F. Gubert:** None. **A.P. Figueirêdo:** None. **A. Torres:** None. **B.D. Paredes:** None. **C. Teixeira:** None. **F. Tovar-Moll:** None. **M.F. Santiago:** None. **R. Mendez-Otero:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.01/A62

Topic: A.09. Adolescent Development

Support: Charles C. Chapelle Fellowship, Purdue University

Title: Chronic adolescent consumption of caffeine mixed alcohol significantly increases ΔFosB expression in the nucleus accumbens

Authors: *M. T. ROBINS, R. M. VAN RIJN;
Purdue Univ., West Lafayette, IN

Abstract: The last decade has seen a shift away from consumption of carbonated beverages to highly caffeinated energy drinks. This is particularly worrying in the adolescent population, where mixing of highly caffeinated beverages with alcohol occurs. As the adolescent brain develops, it is important to understand the potential long-term behavioral and neurochemical consequences of chronic exposure to caffeine mixed alcohol. Previously, we have found that, compared to exposure to caffeine or alcohol alone, adolescent mice exposed to caffeine mixed alcohol exhibit potentially worrying alterations in behavior, like increased locomotor sensitivity, blunted place preference, and impaired cognition. Drugs of abuse like cocaine, alcohol, and caffeine enhance dopamine release to neurons in the dorsal striatum and nucleus accumbens through mesolimbic and nigrostriatal projections. Chronic activation of these neurons can increase ΔFosB expression, a transcription factor implicated in long-term neuronal plasticity. It is unclear if the combination of caffeine and alcohol produces cumulative or synergistic release of dopamine and would cause significant increases in ΔFosB expression. We hypothesized that chronic adolescent exposure to caffeine mixed alcohol would increase ΔFosB expression in the dorsal and ventral striatum, similar to that observed for chronic cocaine exposure. To test our hypothesis, we chronically exposed mice to water, caffeine, alcohol, or caffeine mixed alcohol throughout adolescence. Afterwards, mice were sacrificed and immunohistochemistry was used to quantify ΔFosB expression in the dorsal striatum and nucleus accumbens. ΔFosB expression in the dorsal striatum increased in animals exposed to caffeine mixed alcohol compared to water treated mice, however no significant difference was observed between animals exposed to caffeine versus caffeine mixed alcohol. Yet, ΔFosB expression in nucleus accumbens was significantly increased in mice chronically exposed to caffeine mixed alcohol compared to caffeine or alcohol alone. How this increase in ΔFosB expression in the nucleus accumbens correlates with our behavioral data remains to be investigated. Overall, our data suggests that chronic exposure to caffeine mixed alcohol, unlike caffeine or alcohol alone, selectively increases ΔFosB expression levels in the nucleus accumbens and mimics cocaine-induced increases in ΔFosB expression in this particular brain area, known to be critical for drug reward behavior.

Disclosures: **M.T. Robins:** None. **R.M. van Rijn:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.02/A63

Topic: A.09. Adolescent Development

Support: NIH Grant DA029815

Title: The effects of amphetamine on synaptic plasticity in the medial prefrontal cortex are more pronounced in adolescent- compared to adult-exposed rats

Authors: *S. KANG[1,2], C. L. COX[4], J. M. GULLEY[2,3];
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Abstract: High frequency stimulation (HFS) in superficial layers of the rat medial prefrontal cortex (mPFC) tends to induce long-term depression (LTD) *in vitro*. The mechanism for this may be a stimulation-induced increase in inhibitory transmission, rather than an attenuation of excitatory transmission. Moreover, it may be modulated by dopamine receptors that are known to be prevalent in the mPFC. In the current study, we investigated the mechanisms underlying HFS-induced LTD in the mPFC and whether amphetamine (AMPH) exposure would disrupt this plasticity. In experiment 1, naïve male Sprague Dawley rats were sacrificed during mid-adolescence [around postnatal day (P) 35] and young adulthood (around P70 and P125). Slices of their mPFC were prepared for *in vitro* field potential recordings. Four trains of 50-Hz HFS were delivered in the superficial layers of the mPFC to trigger LTD-like plasticity in deep layer neurons. Subsequently, we used various bath-applied drugs to examine the role of GABAA, dopamine, NMDA and metabotropic glutamate receptors in the formation of this plasticity. Our preliminary results suggests that HFS-induced LTD in the mPFC differs in adolescents compared to adults and is dependent on dopamine receptor activity. In experiment 2, rats were pre-treated with 3 mg/kg AMPH or saline (i.p.) every other day from P27 to 45 or P85 to 103 and HFS-induced plasticity was measured *in vitro* 3-4 weeks after the last injection. Our preliminary results suggest that HFS triggers LTD in controls, but it triggers LTP-like responses in AMPH-exposed rats. This effect was more pronounced in rats exposed during adolescence. These results are consistent with our previous finding that chronic AMPH exposure dampens inhibitory transmission in deep layer mPFC cells and further demonstrate a potential heightened vulnerability to drug-induced plasticity during adolescence. We hypothesize that this persistent AMPH-induced dysregulation of inhibitory tone in the mPFC may lead to a "noisy" PFC output and contribute to observed deficits in cognition in AMPH-exposed individuals.

Disclosures: S. Kang: None. **C.L. Cox:** None. **J.M. Gulley:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

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Topic: A.09. Adolescent Development

Support: F31 DA036330

R01 DA029815

Title: Age- and sex-dependent differences in acquisition and persistence of methamphetamine self-administration

Authors: *E. R. HANKOSKY[1], R. M. HAAKE[1], A. R. GOLD[1], E. C. KROEGER[1], J. M. GULLEY[1,2];
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Abstract: Individuals who initiate drug use during adolescence, and those who are female, tend to have more adverse long-term consequences than adult-onset users and males. Here, we used rats to investigate age- and sex-specific differences in acquisition and persistence of methamphetamine (METH) self-administration (SA). Male and female Sprague-Dawley rats were assigned to either adolescent- or adult-onset SA groups and implanted with an indwelling catheter in the right jugular vein [on postnatal days (P) 32 or 82, respectively]. Starting on either P40 or P90, rats began training and testing that lasted for 24 days. Acquisition of SA was assessed at 0.05 mg/kg/inf METH under fixed ratio 1 (FR1), FR3, and FR5 schedules, followed by FR5 testing with two within-session periods (15 min each) where illumination of the house light was used to signal that drug was not available (9 sessions total). Our preliminary results indicate that adult-onset females exhibit heightened acquisition of METH SA, but that adolescent- and adult-onset males are more persistent in METH seeking during periods of signaled non-availability. Control adolescent- and adult-onset counterparts that self-administered saccharin (SACC; 0.1%) demonstrated robust age- and sex-specific differences, such that adult-onset females acquired most readily and maintained the highest levels of responding. All groups learned to reduce responding when SACC was unavailable. These findings suggest that females and adolescents display heightened acquisition and persistence, respectively, which are distinct patterns of drug-seeking that may lead to poorer outcomes following repeated use. Furthermore, the age and sex-specific characteristics of non-drug reward seeking are considerably different than those observed for METH, which highlights the distinct aspects of METH SA in adolescent and adult rats.

Disclosures: **E.R. Hankosky:** None. **R.M. Haake:** None. **A.R. Gold:** None. **E.C. Kroeger:** None. **J.M. Gulley:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.04/A65

Topic: A.09. Adolescent Development

Support: Northeastern TIER 1 Grant

Title: Can environmental enrichment protect against oxidative stress, parvalbumin loss, and behavioral deficits after early life stress? A preliminary study

Authors: V. THOMPSON[1], C. DO PRADO[3], H.-N. LEE[1], F. HOLLAND[1], *H. C. BRENHOUSE[2];
[2]Psychology, [1]Northeastern Univ., Boston, MA; [3]PUCRS, Porto Alegre, Brazil

Abstract: Exposure to early life stress (ELS) alters development, which purportedly yields behavioral deficits and vulnerability to mental illness in adolescence. For example, ELS causes cognitive deficits in adolescence that co-occur with loss of parvalbumin-expressing interneurons in the prefrontal cortex. Importantly, parvalbumin-expressing interneurons play an important role in cognitive function, and are particularly vulnerable to damage via oxidative stress early during early postnatal development. Environmental enrichment (EE) has been shown to buffer several effects of ELS, however the mechanism underlying the protective effects of EE is not well understood. Therefore, here we investigated whether ELS increases oxidative stress in the prefrontal cortex, and whether EE can prevent oxidative stress, parvalbumin loss, and cognitive deficits. Male rats (n=4) were exposed to control rearing or maternal separation ELS from postnatal days 2-20, followed by rearing from weaning through adolescence in either EE (large cage and social groups with novel objects and climbing opportunities) or standard housing. In adolescence (postnatal day 55), rats were tested for cognitive performance using the win-shift paradigm, followed by Western blot analysis of the prefrontal cortex for parvalbumin and for markers of oxidative stress. EE prevented increased errors on the win-shift paradigm after ELS. Preliminary data suggests that ELS also increased NADPH oxidase 2 (NOX2) in the adolescent prefrontal cortex. However, while EE protected ELS-exposed animals from deficits in win-shift performance, EE did not prevent ELS-induced PVB or NOX2 changes. Taken together, these data suggest that ELS yields increased enduring oxidative stress that likely contributes to parvalbumin loss in adolescence. EE appears to interfere with mechanisms other than oxidative

stress that contribute to cognitive deficits, since EE protected adolescents from cognitive, but not oxidative stress effects of ELS. Future investigations will determine whether EE protects against cognitive deficits via oxidative stress mechanisms in subcortical regions such as the hippocampus, which has been shown to be protected from ELS after EE rearing.

Disclosures: V. Thompson: None. **C. do Prado:** None. **H. Lee:** None. **F. Holland:** None. **H.C. Brenhouse:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

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Program#/Poster#: 32.05/A66

Topic: A.09. Adolescent Development

Support: R01 DA029815

Title: The effects of unit dose on age- and sex-dependent differences in methamphetamine self-administration

Authors: *S. R. WESTBROOK, E. R. HANKOSKY, R. M. HAAKE, M. R. DWYER, J. M. GULLEY;
Univ. of Illinois Urbana-Champaign, Champaign, IL

Abstract: Recent work in our lab suggests there are age and sex differences in methamphetamine (METH) self-administration (SA) when rats are trained with a dose of 0.05 mg/kg/infusion. In this study, we extended these findings by utilizing different training doses in separate groups of rats and subsequently performing a dose-response analysis by changing the available unit dose across sessions. Male and female Sprague-Dawley rats were assigned to either adolescent- or adult-onset groups and implanted with an indwelling catheter in the right jugular vein on either postnatal day 32 or 82. Following 6-8 days of recovery, rats were trained for 15 days to self-administer METH at a dose of either 0.02 or 0.08 mg/kg/infusion. The acquisition period consisted of 7 days of responding on a fixed ratio (FR) 1 schedule of reinforcement followed by 4 days each of FR3 and FR5. Following acquisition, motivation to respond for 4 doses of METH (0.02, 0.05, 0.08, 0.1 mg/kg/infusion) was assessed in 4 separate sessions (5 h duration) using a progressive ratio (PR) schedule of reinforcement. Our preliminary results suggest that adolescent-onset females acquire METH SA more readily than adolescent-onset males at both training doses (0.02 and 0.08 mg/kg/infusion). However, rates of acquisition in adolescent-onset males and females were significantly lower at the 0.02 mg/kg/infusion dose

compared to the 0.08 mg/kg/infusion dose. Furthermore, adolescent-onset females worked significantly harder for METH under a PR schedule for all doses tested, regardless of training dose. Investigation of the adult-onset male and female groups is currently in progress. These preliminary findings suggest that at multiple doses, adolescent-onset females will work harder and self-administer more METH than their male counterparts. This heightened METH seeking could contribute to heightened vulnerability to METH addiction in females compared to males.

Disclosures: S.R. Westbrook: None. **E.R. Hankosky:** None. **R.M. Haake:** None. **M.R. Dwyer:** None. **J.M. Gulley:** 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.; Abbott Nutrition.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: Canadian Institutes of Health Research

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Title: DCC receptors control the development of fine axonal structure of mesocortical dopamine neurons and determine behavioral inhibition in adult mice

Authors: *L. M. REYNOLDS[1,3], M. WODZINSKI[3], C. MANITT[3], E. NESTLER[4], C. FLORES[3,2];
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Abstract: The guidance cue netrin-1 and its receptor, DCC, are highly and conspicuously expressed in the mesocorticolimbic dopamine system throughout life. We have recently shown that DCC signaling within dopamine neurons controls the extent of dopamine input to the medial

prefrontal cortex (mPFC) during adolescence. In turn, DCC organizes mPFC local circuitry, significantly influencing cognitive flexibility in adulthood. How precisely DCC receptors control mPFC dopamine input expansion remains to be determined. Here, we used cre-lox recombination to induce *dcc* haploinsufficiency selectively in dopamine neurons ($dcc^{lox/+}DAT^{Cre}$ mice). We then performed quantitative analysis of the architecture of individual mPFC dopamine axons across cingulate 1 (Cg1) and prelimbic (PrL) subregions. To this end, we injected bilaterally an AAV-DIO-eYFP viral vector in the ventral tegmental area (VTA) of adult $dcc^{lox/+}DAT^{Cre}$ or control $dcc^{+/+}DAT^{Cre}$ littermates to label small subsets of mPFC-projecting dopamine axons with eYFP. We find that mPFC dopamine axons of $dcc^{lox/+}DAT^{Cre}$ mice have less complex arbors than those of $dcc^{+/+}DAT^{Cre}$ mice. Furthermore, dopamine axon arbors of $dcc^{lox/+}DAT^{Cre}$ mice are significantly shorter and have fewer varicosities than dopamine axon arbors of $dcc^{+/+}DAT^{Cre}$ littermates. We are now examining whether these less complex dopaminergic axons were destined to innervate limbic regions, but instead were rerouted to the mPFC due to changes in DCC signaling. To assess whether altered organization of mPFC dopamine connectivity is associated with changes in behavioral inhibition, we tested adult $dcc^{lox/+}DAT^{Cre}$ mice in a go/no-go behavioral task we adapted for use in mice. We find that $dcc^{lox/+}DAT^{Cre}$ and wild-type mice show similar acquisition of the go/no-go task and similar number of omission errors. However, $dcc^{lox/+}DAT^{Cre}$ mice have improved behavioral inhibition, indicated by fewer commission errors than wild-type controls.

Disclosures: L.M. Reynolds: None. **M. Wodzinski:** None. **C. Manitt:** None. **E. Nestler:** None. **C. Flores:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

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Topic: A.09. Adolescent Development

Support: NIH grant DA009079

Title: Caffeine potentiates cocaine effects on dopamine signaling more in adolescent than adult rat ventral striatum

Authors: *Q. D. WALKER, M. PUSTJOVESKY, C. M. KUHN;
Pharmacol. and Cancer Biol., Duke Univ., Durham, NC

Abstract: Caffeine use rates by adolescents is concerning and little is known about whether they are particularly sensitive to caffeine effects. Caffeine's psychostimulant effects are mediated primarily via antagonism of adenosine receptors. Adenosine A1A and A2A receptors densely populate the striatum in a variety of loci, usually in combination with DA receptors. Adenosine A1A receptors are located on presynaptic dopamine (DA) terminals where they couple with D2 receptors and inhibit DA release. We have observed that caffeine increases locomotor behavior more in adolescent than adult male rats. To resolve a presynaptic contribution to this effect these studies have examined A1A dependent DA release regulation using voltammetry. DA transient frequencies were the same at baseline in adolescent and adult male rats (15.5 and 17.5/min, respectively.) but became significantly greater in adolescents than adults (22 vs. 14/min) from 15 to 30 min following 25 mg/kg caffeine. DA transient duration and amplitude were the same before and after caffeine. Caffeine potentiated the effects of 15 mg/kg cocaine injected 30 mins following caffeine. Caffeine doubled the cocaine-induced increase of transient amplitude in adult rats but the increase in adolescents was almost 6-fold over baseline amplitude. Caffeine enhanced the cocaine-induced increase of DA transient duration by about 2-3 fold in adults and adolescents, respectively. Concurrent changes in tonic extracellular DA concentrations were also determined using principal components analysis. Caffeine decreased tonic DA in both age groups at 5 mins post injection; the decrease was brief in adolescents but sustained in adults. Subsequent injection of cocaine produced greater increases in tonic DA than cocaine alone. Cocaine-induced increases in tonic DA were sustained longer following caffeine in both ages. In contrast to these age differences in adenosine regulation of DA release, D2 autoreceptor antagonism with raclopride induced greater effects on phasic and tonic DA in adults. Thus, DA release autoregulation shifts developmentally from primarily adenosine A1 in adolescence to DA D2 in adulthood. Human adolescents may be particularly vulnerable to the ability of caffeine to increase the rewarding value of other drugs of abuse.

Disclosures: **Q.D. Walker:** None. **M. Pustjovesky:** None. **C.M. Kuhn:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.08/A69

Topic: A.09. Adolescent Development

Support: NIMH R01-MH086507

Title: Transient NMDAR blockade during adolescence selectively disrupts the frequency-dependent heterosynaptic inhibition of basolateral amygdalar transmission in the adult prefrontal cortex

Authors: *D. R. THOMASES[1], S. BELTON[2], K. Y. TSENG[1];
[1]Cell. and Mol. Pharmacol., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL;
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Abstract: While much of the brain is mature by the time of adolescence, the prefrontal cortex (PFC) continues to undergo major functional changes during this transitional period that depend upon NMDAR-mediated transmission. We have recently shown that adolescent exposure to the non-competitive NMDAR antagonist MK-801 produces an enduring frequency-dependent disruption of prefrontal local field potential (LFP) responses to ventral hippocampal drive that was not seen when MK-801 was given during adulthood. Here we examined whether a similar age-dependent PFC dysregulation occurs with other inputs such as those originated from the basolateral amygdala (BLA). We found that the overall pattern of BLA-evoked LFP facilitation and inhibition in the PFC remains unaltered following adolescent MK-801 treatment (0.1 mg/kg, i.p., from postnatal day 35 to 40). Both saline and MK-801-treated rats exhibited similar degrees of BLA-evoked paired-pulse facilitation of the homosynaptic response at 10 and 20 Hz, and LFP suppression at 40 Hz. Interestingly, a disruption of the heterosynaptic regulation of BLA-evoked LFP emerged following adolescent MK-801 treatment. Typically, the pattern of LFP facilitation and inhibition in saline controls is no longer present when the BLA-evoked LFP is preceded by a heterosynaptic response elicited from the ventral hippocampus. While the hippocampal-induced heterosynaptic modulation of the BLA response was not apparent at 10 Hz, it did suppress the amplitude of BLA-evoked LFP at 20 Hz and increased the degree of LFP suppression at 40 Hz. Such frequency-dependent inhibitory regulation of the BLA response by the ventral hippocampus is lacking in the PFC of adult rats that received MK-801 treatment during adolescence. Collectively, these results point to an interesting possibility that NMDAR-mediated function during adolescence is essential for the functional maturation of a heterosynaptic mechanism of inhibitory control in the PFC that can be driven by the ventral hippocampus to suppress BLA inputs. A disruption of PFC afferent processing could extend to impair the inhibitory regulation of other inputs and contribute to widespread and enduring prefrontal-dependent cognitive deficits later in life.

Disclosures: D.R. Thomases: None. **S. Belton:** None. **K.Y. Tseng:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

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Program#/Poster#: 32.09/A70

Topic: A.09. Adolescent Development

Support: NIH Grant DA029815

Title: Effects of amphetamine exposure during adolescence on orbitofrontal cortex neurons that encode goal-directed and habitual behaviors

Authors: *L. R. HAMMERSLAG[1], A. J. CONTRERAS-ROGERS[2], P. B. SHAH[2], A. N. MARKS[2], J. M. GULLEY[2];
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Abstract: Human drug users characteristically engage in habitual behavior, such as continuing to seek and use drugs in the face of diminished value. Similarly, animals that have been exposed to psychostimulants display more rapid development of habits as well as deficits in adapting to new information about reward contingencies and value. The orbitofrontal cortex (OFC) may mediate the effects of drug exposure on habit formation, as OFC-lesioned animals resemble drug-exposed animals in tests of habitual responding. Here, we used *in vivo* electrophysiology to examine OFC activity following outcome devaluation in rats exposed to amphetamine (AMPH) or saline during adolescence. Male and female Sprague-Dawley rats were exposed to AMPH every other day during adolescence (postnatal days 27-55). After a 3-week withdrawal period, microwire electrodes were surgically implanted into the OFC and recordings were obtained during behavior sessions where rats responded for a reinforcer under random interval (RI) 30 schedule and during extinction sessions that followed 1-h ad libitum access to the reinforcer (outcome devaluation). Neurons that displayed lever press (LP)-related activity were characterized as up- or down-modulated and a modulation rate (maximum difference from baseline) was calculated for each period of LP-related activity. Preliminary results suggest that the majority of LP-related neurons are up-modulated during RI30 training sessions and when the reinforcer is devalued. Furthermore, up-modulated neurons have a greater positive modulation rate during the devalued test relative to the RI30 sessions. During the valued test, however, the majority of lever-press related neurons are down-modulated, resulting in a negative modulation rate. Data collection is ongoing but we hypothesize that AMPH-exposed animals will be more habit-based compared to controls and that OFC neurons will encode this drug-induced change in behavior. It is important to clarify the relationship between habitual behavior and OFC function in drug-exposed animals as it is currently unclear if drug-exposure alters how the OFC responds to changes in value, or if drug-exposure simply reduces OFC activity non-specifically.

Disclosures: **L.R. Hammerslag:** 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.; Abbott Nutrition. **A.J. Contreras-Rogers:** None. **P.B. Shah:** None. **A.N. Marks:** None. **J.M. Gulley:** 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.; Abbott Nutrition.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.10/A71

Topic: A.09. Adolescent Development

Support: R01 MH099118-01A1, National Institute of Mental Health (NIMH)

Title: Effect of peridolescent dopaminergic perturbation on adult dopamine circuit activity and dopamine-regulated behaviors

Authors: *D. SURI[1], N. CHUMA[2], M. ANSORGE[3];
[1]Developmental Neurosci., NYS Psychiatric Inst., New York, NY; [2]Psychiatry, [3]Developmental Neurosci., Columbia Univ., New York, NY

Abstract: Peridolescence (PA) constitutes a vulnerable period in humans, during which a range of psychiatric disorders as well as drug abuse emerge. We have identified a dopamine (DA) sensitive period in PA that alters aggression and behavioral response amphetamine (AMPH) in rodents. Specifically, enhanced DA levels during P32-P41 achieved by transient blockade of the dopamine transporter (DAT) result in enhanced adult aggressive behavior and sensitized response to AMPH challenge. Hyperactivity of the DA system is associated with increased impulsive aggression, and altered amphetamine response is an indication for perturbed DAergic function. We therefore examined how PA DAT blockade impacts adult DAergic activity and DA-regulated behaviors namely, impulsivity, motivation and working memory. We find that in animals with PA DAT blockade VTA but not SNc DA neurons exhibit enhanced excitability in slice recordings, and increased population activity and burst firing *in vivo.* These results support our hypothesis of a hyper-DAergic phenotype evoked by PA DAT blockade. Concomitant with increased VTA DAergic activity we also note enhanced motor impulsivity in the go-nogo task. These behavioral changes are however not accompanied by alterations in motivation or working memory. This study furthers our knowledge of how perturbed DAergic function during PA impinges on adult DAergic circuit activity to impact complex behaviors implicated in psychiatric disorders and drug abuse.

Disclosures: **D. Suri:** None. **N. Chuma:** None. **M. Ansorge:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.11/A72

Topic: A.09. Adolescent Development

Support: MEDCEN Foundation Grant

Title: Mouse strain differences in midbrain dopamine neuron firing during adolescence

Authors: ***A. PLACZEK**, J. LOGUE, M. SIMMONS;
Mercer Univ. Sch. of Med., Macon, GA

Abstract: In humans, adolescence is a developmental period of increased vulnerability to substance abuse and drug addiction. Those who use drugs and alcohol during adolescence are more likely to develop lifelong problems with addictive substances when compared to adult users. Human data have been consistently supported by animal models which have identified a role for ongoing neuronal development as a key component of the adolescent critical period for drug and alcohol addiction. The mesocorticolimbic dopamine (DA) systems are particularly important in the brain's response to addictive drugs, and all known addictive substances increase DA release from neurons originating in the ventral tegmental area (VTA). 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 nucleus accumbens (NAc). Our previous work has shown that direct depolarization of ventrolateral VTA DA neurons leads to greater sustained action potential firing in adolescent C57BL/6J mice compared to adults. Given the fact that C57BL/6J mice are known as an "alcohol-preferring" strain, and that these mice are also known to engage in binge-like alcohol consumption during adolescence, we wondered if an "alcohol non-preferring" strain (FVB/NJ) showed similar firing patterns during adolescence. We conducted patch-clamp recording in horizontal brain slices containing the VTA, measuring depolarization-induced burst durations in current-clamp mode of periadolescent (5 week old) and adult (3-6 month old) mice of both strains. We found again that the VTA DA neurons of periadolescent C57BL/6J mice fired significantly longer action potential trains than those of adults, however, this was not the case for those of FVB/NJ mice. In fact, adolescent FVB/NJ neuronal firing was not significantly different from adults, suggesting that the adolescent firing difference may be linked to alcohol preference and possibly binge-like consumption. We previously showed that increased activity of

small-conductance calcium-activated potassium (SK) channels was responsible for increased action potential firing durations in adolescent C57BL/6J mouse VTA DA neurons. We therefore examined apamin-sensitive, SK-mediated tail currents in adolescent FVB/NJ mice, and compared them to those of adults. Our findings suggest that decreasing SK channel function may reduce binge-like alcohol consumption in adolescent, alcohol-preferring mice, while increasing SK channel activity may have the opposite effect in alcohol non-preferring mice.

Disclosures: **A. Placzek:** None. **J. Logue:** None. **M. Simmons:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

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Program#/Poster#: 32.12/A73

Topic: A.09. Adolescent Development

Support: NIH-NINDS P50 NS22343

Title: Communication during a dyadic social interaction in school-age children with high functioning autism and children with williams syndrome

Authors: ***P. T. LAI**[1], M. IGNACIO[2], M. B. KIM[3], U. BELLUGI[3], J. REILLY[2];
[1]Salk Inst. LCN-B, La Jolla, CA; [2]Psychology, San Diego State Univ., San Diego, CA; [3]Lab. for Cognitive Neurosci., Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Social communication is central to the human experience and affects all aspects of one's life. Lacking the ability to convey social information can lead to deficits in maintaining relationships and academic problems in school. The majority of studies investigating how children communicate have focused on children under the age of 7. The goal of this study is to better define the communicative phenotype of school age children in typically developing (TD; n = 26) children, children with High Functioning Autism (HFA; n = 23), and children with Williams Syndrome (WS; n = 14), by analyzing linguistic output during an interaction with an adult. Language was examined by assessing morphological proficiency and syntactic complexity. The social elements of language were assessed through the Reilly Evaluation Coding System (Reilly et al.,1998; 2004). Specifically, social evaluations (i.e., attributing emotions, building suspense, intensifiers, etc.) are measures used to attract and maintain the listener's attention through the narrator's attitude or perspective. Finally, a specific social aspect of the interaction was examined through questions that are asked by the child towards the experimenter. For the rates of morphsyntactic errors, differences were observed ($p<.001$); the HFA and WS groups

produced more morphosyntactic errors than the TD group. Next, differences were observed for syntactic complexity (p=.006); the TD group produced more complex syntax than both HFA and WS groups. However, to assess how children use language for social purposes, the HFA group produced fewer of these evaluations (p=0.09) than either the WS or TD groups. Finally, children asking questions were examined. This behavior was observed in 2 out of 26 TD children, 12 out of 23 HFA children, and 10 out of 14 WS children. A content analysis was then completed. For the TD group, only 2 questions were asked. Of the 29 questions asked by the HFA children, 8 were personal and 21 were on the topic of discussion. The WS group posed 25 questions, 12 personal and 13 on the topic of discussion. An example of a question asked from a WS child: "where did you go to dinner". Taken together, the language results suggest the structure and quality of language in both the HFA and WS children are not on par with their TD peers. When examining evaluative devices that serve social purposes, the WS group are more expressive followed by the TD group then HFA group. Asking questions during the interaction was a rare behavior in the TD group, while the HFA and WS groups posed numerous questions, but their content differed. These specific behaviors may suggest the WS individuals are trying to engage the experimenter into the conversation.

Disclosures: P.T. Lai: None. **M. Ignacio:** None. **M.B. Kim:** None. **U. Bellugi:** None. **J. Reilly:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.13/A74

Topic: F.03. Motivation and Emotion

Support: Wellcome Trust PhD studentship

Title: Evidence that behavioural phenotypic effects of increased 5-HT transporter expression are mediated by a neurodevelopmental mechanism

Authors: *A. SENGUPTA[1], S. MCHUGH[2], A. TAYLOR[2], T. SHARP[3], D. BANNERMAN[2]; [2]Dept. of Exptl. Psychology, [3]Dept. of Pharmacol., [1]Univ. of Oxford, Oxford, United Kingdom

Abstract: Variation in the expression of the 5-HT transporter (5-HTT) has been linked to altered emotionality. For instance, prior research has shown that both humans and rodents with higher levels of the 5-HTT exhibit a phenotype of reduced anxiety and fear, though the mechanisms through which this is mediated remain unclear. One theory suggests that altered 5-HT

transmission specifically during development gives rise to the adult phenotype. To investigate this hypothesis, we studied ethological unconditioned anxiety and fear learning in early adolescent (P25 - P40) 5-HTT overexpressing (5-HTTOE) mice. We used a battery of tests including the elevated plus maze, hyponeophagia, successive alleys, black-white alley, open field test, and a standard cue-based fear-conditioning paradigm. The results taken across a range of tests indicated that young 5-HTTOE mice already have an established phenotype of reduced anxiety and fear, which is similar to that observed previously in adult 5-HTTOE mice. This phenotype could be driven either by discrete developmental changes in 5-HT signalling or by the ongoing activity of the 5-HTT at the time of the behaviour. To test the latter, citalopram (10 mg/kg) was administered acutely during the training session of the fear conditioning task. It was confirmed that fear learning was decreased in early adolescent 5-HTTOE mice compared to wild-type mice. Moreover, the deficit in fear learning of 5-HTTOE mice was not rescued by the acute dose of citalopram. Thus, the phenotype in the 5-HTTOE mice does not appear to be mediated solely by overexpression of the 5-HTT at the time point at which the behavioural task is performed. These findings support the idea that developmental events, rather than ongoing activity of the 5-HTT, are the underlying mechanism for the phenotype of reduced fear observed in 5-HTTOE mice.

Disclosures: **A. Sengupta:** None. **S. McHugh:** None. **A. Taylor:** None. **T. Sharp:** None. **D. Bannerman:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.14/A75

Topic: E.03. Behavioral Neuroendocrinology

Title: An investigation into the effects of developmental exposure to Bisphenol-S on anxiety in rats

Authors: *H. A. MOLENDA-FIGUEIRA, R. S. KLISH, S. F. KREUL, T. R. BECKER, K. L. FLETTY, S. N. HASEKER;
Psychology Dept., Univ. of Wisconsin-Stevens Point, Stevens Point, WI

Abstract: It has been suggested that developmental exposure to endocrine disruptors may play a role in the development of a diverse group of maladies, ranging from cancers to attention deficit hyperactivity disorder, neurodegeneration and Schizophrenia. This has prompted the removal of some of these chemicals, such as Bisphenol-A (BPA) from commonly-used plastic products.

However, BPA has been replaced with a similar chemical, Bisphenol-S (BPS), which is also known to alter hormone actions in a variety of tissues, potentially leading to illness. Little is currently known about the impact of BPS on health or behavior. We investigated the impact of developmental BPS exposure on anxiety-like behaviors in rats, and whether sex differences in response to BPS treatment are present. Pregnant Sprague-Dawley rats were exposed to BPS in drinking water (1mg/L of water) beginning on gestational day 12. This resulted in a BPS dose of approximately 15µg/kg/day. Control dams received plain water. At 1 day of age, the sex of rat pups was determined and litters were culled resulting in a 2-cohort final total of 10 rats/sex/water treatment group. Exposure to BPS through drinking water continued until 45 days of age. Beginning at 21 days of age (juvenile test), rats received one of 3 anxiety tests: the elevated plus maze (EPM), light-dark box (L-D Box) or open field test (OF). Rats received 1 type of test on alternate days until all 3 tests were completed. Each test was conducted for 5 minutes, and behavior was recorded using video cameras. Rats were again tested for anxiety beginning at 37 days (peripubertal test) and finally beginning at 60 days of age (adult test). Females were monitored for estrous cycle stage via appearance of vaginal cytology, and anxiety tests were only administered on the day of estrus. Duration of time spent in the open versus closed arms for the EPM, in the light versus dark compartments for the L-D Box and time spent in corners, center or sides of the OF test was quantified using a MS-DOS computer program. Preliminary results of Cohort 1 demonstrate an interaction between Sex and Treatment for the EPM, suggesting that control male rats were the most anxious. However, during the peripubertal tests, there was a trend for BPS-treated animals to spend more time in the corners of the OF test, indicating higher levels of anxiety compared to controls. Because BPS treatment was ongoing during the peripubertal time point, this could indicate that while BPS may have organizational effects on the brain early on in development, as has been shown for BPA, puberty may be another sensitive time for not only organization of circuits mediating anxiety but also manifestations of anxious behaviors.

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Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: A.09. Adolescent Development

Support: NIH Grant

Title: Developmental and sex-dependent effects of early life stress on glutamate receptor expression

Authors: *P. GANGULY[1], H. C. BRENHOUSE[2];
[1]Psychology, [2]Northeastern Univ., Boston, MA

Abstract: Exposure to early life stress increases vulnerability to psychiatric disorders such as depression and anxiety, which often develop in adolescence or young adulthood and manifest in a sex-dependent manner. Numerous studies have shown that ELS disrupts normal development of prefrontal cortex (PFC) receptor expression and functional connectivity to the limbic system. ELS may also disrupt glutamate receptor trafficking and mobility, which play an important role in stress and behavior adaptation. However, the mechanisms by which ELS impacts glutamatergic receptor expression in males and females are still relatively unknown. PFC AMPA and NMDA receptors mediate excitatory synaptic transmission and behavior through tightly regulated post-translational trafficking to the membrane. Previous studies have indicated that ELS in the form of maternal separation (MS) leads to overexpression of NMDA receptor (NMDAR) subunit NR2A within the medial PFC. Membrane density of GluR2-containing AMPA receptors (AMPARs) in the PFC is also substantially elevated after acute stress, further suggesting that stress can affect trafficking of NMDARs and AMPARs. Here we compared plasma membrane and cytoplasmic expression of NR2A and GluR2 subunits in the PFC of male and female rats, either reared under control (CON) conditions or exposed to MS. Western blot analyses of membrane or cytoplasmic fractions were performed on animals sacrificed on postnatal day 25 (juvenility) or 40 (adolescence). We observed sex- and developmental related changes in membrane expression of NR2A and GluR2. Specifically, males expressed less NR2A and GluR2 in both cytoplasmic and membrane fractions compared to females. However, MS caused increased membrane NR2A in male adolescents but not female adolescents. These results suggest sex and rearing condition effects on AMPA and NMDA receptor trafficking. One interpretation is that increased insertion of AMPARs and NMDARs during early adolescence in females could facilitate resilience in females, while the absence of such a mechanism could yield males more susceptible to some ELS effects. However, the age-dependent expression of these receptors, along with their subunit composition, will shed further light on possible developmental influences on sex-dependent ELS effects.

Disclosures: P. Ganguly: None. **H.C. Brenhouse:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: A.09. Adolescent Development

Support: NSFC Grant 81401129

Title: Behavioral alterations induced by adolescent chronic social instability stress are correlated with downregulation of nectin-3 in medial PFC in mice

Authors: *J.-T. LI[1], X. WANG[2], H. WANG[1], Y.-A. SU[1], T. SI[1];
[1]Inst. of Mental Health, Peking Univ., Beijing, China; [2]Dept. of Neurobio., Zhejiang Univ. Sch. of Med., Hangzhou, China

Abstract: Background: Adolescence is a critical developmental period witnessing substantial remodeling of neural circuits, especially the prefrontal cortex. Chronic social instability stress in adolescence has been known to induce persistent alterations in a variety of behaviors. However, it remains to be determined how the prefrontal cortex is affected by this paradigm. Methods: Male mice were exposed to chronic social instability stress from postnatal day 28 to 77. One week after treatment, they were tested for anxiety-related behaviors in the open field, social behaviors (including social preference and recognition) and spantaneous alternation in Y maze. At the end of behavioral testing, animals were sacrificed and we measured the expression levels of several synaptic proteins in medial PFC, including synaptophysin, PSD-95, nectin-1 and nectin-3. Result: Social stressed mice exhibited increased anxiety levels, impaired performance in social recognition and spontaneous alternations, whereas social preference and novel object recognition were not affected. Among several synaptic proteins of mPFC, only nectin-3 was found to be significantly decreased in stressed animals. Moreover, the downregulation of nectin-3 significantly correlated with behavioral alterations. Conclusion: These findings add to evidence that chronic social stress during adolescence modifies the PFC development, which may serve as the underlying mechanisms for stress-induced behavioral deficits.

Disclosures: **J. Li:** None. **X. Wang:** None. **H. Wang:** None. **Y. Su:** None. **T. Si:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

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Program#/Poster#: 32.17/A78

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Consumption of a high-fat diet during adolescence results in long-lasting impairments in sensorimotor gating and stress reactivity

Authors: *P. KALYAN-MASIH, J. VEGA-TORRES, T. HEERS, J. D. FIGUEROA;
Loma Linda Univ. Sch. of Med., Loma Linda, CA

Abstract: Adolescence is a critical period in the neurobiological programming of lifelong stress coping mechanisms. The brain undergoes extensive rearrangement during this maturation period, which underscores a unique sensitivity to environmental factors. Dietary-essential omega-3 polyunsaturated fatty acids (n-3 PUFAs) are gaining recognition as potent nutritional factors underpinning optimal brain development and function. However, their consumption is not sufficient in the reported typical "Western" dietary patterns, in particular during adolescence. This study investigates the impact of a Western diet (WD) on stress responses and sensorimotor gating behaviors using the elevated plus maze, the acoustic startle reflex (ASR) and the prepulse inhibition (PPI) of the ASR. Adolescent Lewis rats (PND 28; $n = 36$) were fed *ad libitum* during four weeks in either experimental WD (42% kcal from fat; $n = 18$) or control diet (7 % kcal from fat; $n = 18$). During early adulthood (PND 58), all rats received the same control diet for four additional weeks before behavioral testing. We found that the rats that consumed the WD during adolescence exhibited reduced ASR and PPI responses (approximately 30% reduction) when compared to control animals ($p < 0.05$). The WD had no effect on body weight in the adult rats ($p > 0.05$) Notably, we show that the WD impaired the ability of these animals to cope with stress, as evidenced by a significant increase in anxiety-like behaviors (elevated plus maze, open field test) following exposure to social isolation and predatory threat stress. Collectively, this study shows that the neurobehavioral responses to stress are sensitive to WD intake during adolescence. Understanding how nutrition affects the adolescent brain is critical, as alterations during this period impose vulnerability to stress-associated disorders, including PTSD.

Disclosures: **P. Kalyan-Masih:** A. Employment/Salary (full or part-time):; Loma Linda University School of Medicine. **J. Vega-Torres:** None. **T. Heers:** None. **J.D. Figueroa:** A. Employment/Salary (full or part-time):; Loma Linda University School of Medicine.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

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Program#/Poster#: 32.18/A79

Topic: A.09. Adolescent Development

Support: MH-083728

MH-094268 Silvo O. Conte center

Title: DISC1 mutation in astrocytes synergistically interacts with adolescent cannabis exposure to affect cognitive function in adult mice

Authors: *S. ABAZYAN, B. ABAZYAN, O. MYCHKO, A. SHEVELKIN, C. YANG, A. KAMIYA, M. PLETNIKOV;
Psychiatry, Johns Hopkins Univ., Baltimore, MD

Abstract: Marijuana use during adolescence has been linked to the increased risks for psychoses and long-term cognitive impairment. The literature supports a multi-factorial view of the liability to psychiatric and cognitive disorders. Thus, we assess the effects of adolescent cannabis exposure on cognitive dysfunction in mice expressing mutant Disrupted-In-Schizophrenia-1 (DISC1) in astrocytes that have been recently implicated in the cognitive effects of cannabis. Control and mutant DISC1 mice were treated with saline or tetrahydrocannabinol (THC) (8.0 mg/kg) for 21 days during postnatal days (P) 32-52; and three weeks later mice were tested for novelty-induced hyperactivity in open field, anxiety in elevated plus maze, spontaneous alteration in Y maze, spatial recognition memory in Y maze, novel object recognition, place preference test and fear conditioning. Compared to all other groups, THC-treated DISC1 mice exhibited impaired spatial, object recognition and place recognition memory. These effects of THC were dependent on expression of mutant DISC1 during adolescent exposure and were not observed after adult THC exposure or adolescent exposure to amphetamine. Our findings indicate that chronic adolescent THC exposure leads to cognitive deficits in mice carrying DISC1 mutation in astrocytes and suggest that genetically predisposed individuals are more sensitive to adolescent marijuana use to develop cognitive impairment.

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Poster

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Program#/Poster#: 32.19/A80

Topic: A.09. Adolescent Development

Support: NIH Grant R36 DA038229-01

Title: Age related differences in the neural representation of magnitude discrimination and cognitive impulsivity in the orbitofrontal cortex

Authors: *L. R. AMODEO, J. D. ROITMAN;
Psychology, Univ. of Illinois, Chicago, Chicago, IL

Abstract: The underdevelopment of the prefrontal cortical system in adolescents is thought to explain a relative increase in maladaptive decisions during this period. Specifically, the orbitofrontal region of prefrontal cortex (OFC) has been shown to play a central role in guiding goal-directed decisions. The aim of this research is to assess, across the lifespan, how OFC encodes the cues that predict reward availability and the receipt of immediate and delayed rewards. To this end, microwire electrode arrays were implanted into the OFC of both adolescent and adult rats so that activity of multiple single-neurons could be recorded during behavioral performance. Long-Evans rats were trained to perform a Magnitude Discrimination (MD) task where a choice was given between a small reinforcement lever and a large reinforcement lever, with both rewards delivered immediately. After five sessions of the MD task, rats began a progressive Delay Discounting task (DD), where they were given the choice between two levers: one leading to a smaller sooner (SS) reward and the other to a larger later (LL) reward. The delay to the larger reward for LL presses increased daily from 1s to 5s, 10s, 15s, 20s, 50s, 70s and 90s. We found no behavioral differences between age groups on DD performance. While inconsistent with prior research, this discrepancy may be due to differences in age-dependent learning and engagement in the task. Using the MD task, we matched learning for both ages so that both groups begin the DD task with an equivalent preference for the large reward. Further, animals are required to attend to the task by initiating each trial via center port response. While adolescent and adult rats performed the MD and DD tasks, we recorded the activity of OFC neurons. Despite a lack of behavioral effects, reward and temporal processing during decision-making were neurologically different in adolescents compared to adults. While the behavioral responses follow the same general pattern in adolescents as adults, adolescent rats do not exhibit a phasic response at the time of the behavioral choice and have an overall blunted response at the time of the later reward. Overall, this research provides a novel insight into how orbitofrontal cortex executes impulse control across development, and suggests that adults and adolescents may leverage different neurological mechanisms during impulse control.

Disclosures: L.R. Amodeo: None. **J.D. Roitman:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.20/A81

Topic: A.09. Adolescent Development

Support: NSF IOS 1257679

NIH NIDA R15 DA035478

NIH NIDA RO1 DA019921

NSF IOS 0921874

Title: Adult mesocorticolimbic tyrosine hydroxylase activity is increased by adolescent social defeat

Authors: *M. A. WEBER[1], J. L. SCHOLL[1], R. T. PAULSEN[1], G. L. FORSTER[1], K. J. RENNER[2], M. J. WATT[1];
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Abstract: Adolescent social defeat in rats, a model of teenage bullying, results in decreased medial prefrontal cortex (mPFC) dopamine (DA) activity in early adulthood. Precise mechanisms influencing the defeat-induced decrease in mPFC DA activity are not fully understood, but may involve altered tyrosine hydroxylase (TH; rate-limiting enzyme in DA synthesis) activity. We used two experimental approaches to examine TH activity in specific regions of the young adult brain after adolescent defeat. First, brain tissue collected from previously defeated and control rats in early adulthood (postnatal day [P]56) was processed using western immunoblot for total and phosphorylated serine 40 (pSer40) TH expression. Second, previously defeated and control rats received acute injections of the amino acid decarboxylase (AADC) inhibitor NSD-1015 (100 mg/kg, ip.) at P56. Brain tissue DOPA (DA precursor) accumulation served as a measure of *in vivo* TH activity. Adult mPFC TH activity was increased after adolescent defeat, as indicated by both increased pSer40 TH expression and greater DOPA accumulation 30 min after AADC inhibition. Within subcortical regions, AADC inhibition elicited greater DOPA accumulation after 180 min in the striatum of previously defeated rats, indicating higher *in vivo* TH activity, with a similar effect in the nucleus accumbens (NAc) core after 30 min. No differences in DOPA accumulation as a result of adolescent defeat were evident in the NAc shell or the ventral tegmental area. Increased adult mPFC TH activity after adolescent defeat is contrary to our initial hypothesis that TH activity would be decreased to result in lower mPFC DA activity. We speculate that increased mPFC TH activity may be acting as a compensatory mechanism attempting to raise mPFC DA synthesis. Current studies are examining TH activity 180 min after AADC inhibition, along with western immunoblots to measure total and pSer40 TH expression in the affected subcortical regions. Findings to date suggest that adolescent defeat has long-lasting effects on TH activity in distinct cortical and

subcortical regions, implying alterations to DA synthesis. However, the functional significance of the subcortical changes to TH activity is unknown.

Disclosures: **M.A. Weber:** None. **J.L. Scholl:** None. **R.T. Paulsen:** None. **G.L. Forster:** None. **K.J. Renner:** None. **M.J. Watt:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.21/A82

Topic: F.03. Motivation and Emotion

Support: NIH R15MH102717

Title: The novel endocannabinoid degradation inhibitor, MJN110, dose-dependently alters social behavior and medial prefrontal cortex activation in adolescent male rats

Authors: *R. L. JONSCHER[1], E. E. BOXER[2], E. C. LOETZ[2], A. ALESSI[2], M. T. ISHIKI[3], S. T. BLAND[2];
[2]Psychology, [3]Biol., [1]Univ. of Colorado-Denver, Denver, CO

Abstract: The endocannabinoid (eCB) system of the brain is involved in many social behaviors and cognitive processes, and may be involved in mood disorders including anxiety. Here, we tested the hypothesis that enhancing the eCB 2-arachidonylglycerol (2-AG) would increase pro-social behavior in the social interaction test, suggesting decreased anxiety. Furthermore, we proposed that the decrease in anxiety would be associated with altered activation of parvalbumin-expressing GABAergic interneurons in the basal-lateral amygdala (BLA) and medial prefrontal cortex (mPFC). We administeredMJN110, which inhibits monoacylglycerol lipase, the enzyme that degrades 2-AG. After 2 hr, adolescent male rats were exposed to a 15 min encounter with a novel conspecific. 90 min later brains were removed and processed for immunohistochemistry (IHC). We observed dose-dependent alterations in social behaviors produced by MJN110. Low doses of MJN110 significantly increased play behaviors in adolescent male rats during a social encounter, whereas a high dose produced a significant reduction in total social interaction. These observations suggest that increased 2-AG signaling may elicit an anxiolytic response at a low dose and sedative effects at a high dose. Double-label IHC for Fos and parvalbumin revealed that MJN110 leads to dose-wise, regionally specific alterations in the activation of parvalbumin-positive cells. At a high dose (5mg/kg), double labeled cells were reduced in the mPFC, but not the BLA. These results suggest that mPFC, but

not BLA mediates 2-AG's effects on play and anxiety-like behaviors, and imply that the behavioral effects of MJN110 may be due to dose-dependent disinhibition of the mPFC.

Disclosures: R.L. Jonscher: None. **E.E. Boxer:** None. **E.C. Loetz:** None. **A. Alessi:** None. **M.T. Ishiki:** None. **S.T. Bland:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.22/A83

Topic: F.03. Motivation and Emotion

Support: NIH Grant R15MH102717

Title: Monoacylglycerol lipase (MAGL) inhibition differentially alters phosphorylation of mTOR in medial prefrontal cortex neurons and astrocytes in adolescent rats

Authors: *E. C. LOETZ[1], R. JONSCHER[2], E. BOXER[2], Z. NARROWE[2], B. GREENWOOD[2], S. T. BLAND[2];
[2]Psychology, [1]Univ. of Colorado Denver, Denver, CO

Abstract: The mammalian target of rapamycin (mTOR) is a protein kinase that has been identified in neurons and glial cells and has a role in plasticity through regulation of protein synthesis. Activation of mTOR through phosphorylation (pmTOR) can be mediated through numerous extracellular signals, but it is unknown what the endocannabinoid (eCB) system's effect on mTOR phosphorylation might be. 2-arachidonoylglycerol (2-AG) is one of the primary eCBs present in the brain, and is broken down largely by the enzyme MAG-L. The novel compound MJN110 is a potent MAG-L inhibitor capable of increasing central 2-AG levels. We have observed that MJN110 dose-dependently alters social behavior and neuronal activation in the medial prefrontal cortex (mPFC). Here, differing doses of MJN110 were administered systemically to adolescent male rats prior to a single social encounter. pmTOR expression was assessed in neuronal and glial cell types (based on cell morphology) using immunohistochemistry (IHC) in both the prelimbic (PL) and infralimbic (IL) regions of the mPFC. Similar patterns of pmTOR expression were observed in PL and IL. In vehicle-treated rats only, a social encounter increased glial pmTOR expression. A high dose of MJN110 produced a robust decrease in glial pmTOR expression, while neuronal pmTOR expression increased approximately twofold in neurons at this dose. Double-label fluorescent IHC revealed

that pmTOR was expressed in astrocytes but not microglia. These results suggest that 2-AG has opposite and dose-dependent effects on mTOR phosphorylation in neurons and astrocytes.

Disclosures: **E.C. Loetz:** None. **R. Jonscher:** None. **E. Boxer:** None. **Z. Narrowe:** None. **B. Greenwood:** None. **S.T. Bland:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R15MH102717

NIH Grant R25NS080685

Title: Effect of monoacylglycerol lipase (MAGL) inhibition on aggression in female rats after postweaning social isolation

Authors: J. FONTENOT[1], H. HAMIDU[2], M. ISHIKI[2], E. LOETZ[1], *S. T. BLAND[1];
[1]Dept of Psychology, [2]Dept of Biol., Univ. of Colorado, Denver, Denver, CO

Abstract: Early life adversity, including during adolescence, can lead to alterations in normal brain development and can increase susceptibility to behavioral disorders in adulthood. Post-weaning social isolation (PSI) is a model of early life adversity in social animals, and previous work has shown that after PSI, female rats display increased aggression during a social encounter with a novel conspecific. The endocannabinoid system, which includes 2-arachidonoyl glycerol (2-AG), is known to regulate central pathways that are important for emotional regulation. The novel drug MJN110 increases the concentration of 2-AG by inhibiting MAGL, the enzyme responsible for its breakdown. The present study examined the impact of MJN110 on PSI-induced aggression in female rats. Female rats were either group housed (GP) or isolation housed (PSI) for 3 weeks between postnatal days 21 and 42. MJN110 (1 or 5 mg/kg IP) or vehicle was administered 2 hr prior to a single social encounter. Social behaviors and total social interactions were assessed. PSI females displayed more aggressive grooming and more social interaction than GRP females. Both doses of MJN110 decreased aggressive grooming regardless of housing conditions but did not significantly decrease overall social interaction. Thus, 2-AG may selectively modulate aggressive behavior in female rats.

Disclosures: **J. Fontenot:** None. **H. Hamidu:** None. **M. Ishiki:** None. **E. Loetz:** None. **S.T. Bland:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.24/A85

Topic: A.09. Adolescent Development

Title: Effects of paradoxical sleep deprivation on adolescent mice

Authors: *L.-H. TUAN[1], L.-J. LEE[1,2,3];
[1]Grad. Inst. of Anat. and Cell Biol., [2]Grad. Inst. of Brain and Mind Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Sleep insufficiency has become a serious health issue. In modern society, the majority of citizen, including the adolescence, do not obtain enough sleep. Since adolescence is a critical period for brain development, the consequences of insufficient sleep during adolescence should be concerned. In this study, we used five weeks old male C57/BL6 mice to examine the acute effects of 72-hour paradoxical sleep deprivation (SD). The modified multiple platform method was adopted. For mice kept in the home cage and on big platforms, sleep time was not limited and used as controls. Mice of SD and control groups were examined in behavioral, neurochemical and histological aspects. Unlike the results of SD in adult animal models, our results showed minimal changes in adolescent mice immediately after 72-hour SD.

Disclosures: **L. Tuan:** None. **L. Lee:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.25/A86

Topic: A.09. Adolescent Development

Title: Altered plasma sulfate and eaat3 expressions in a mouse model of autism, BTBR T+Itpr3tf/J (BTBR)

Authors: *S.-H. KIM, M. HILGART, A. WADOOD, R. SHYTLE;
Dept. of neurosurgery and brain repair, Univ. of South Florida, Tampa, FL

Abstract: Etiology of autism is unknown due to heterogeneity of the disorder, it has been suggested that genetic variations and various environmental factors may play a synergistic role in autism manifestation. Sulfate may be an important element promoting autism phenotype, especially during early development. Sulfate conjugation of hormones and neurotransmitters is necessary for normal growth and development as well as detoxification of xenobiotics. Recently, it has reported that autistic children have lower sulfate levels and increased oxidative stress markers, suggesting that lower plasma sulfate levels may lead to accumulation of toxins and decreased antioxidant capacity in this population. Increased oxidative stress in autistic individuals has been reported. Glutathione (GSH) plays essential roles in detoxification and balancing redox homeostasis in brain. Cysteine (Cys) is the rate limiting factor in GSH de novo synthesis, thereby performing an essential role in cellular protection against oxidative stress. In neurons, the majority of Cys is transported from extracellular space via excitatory amino acid transporter 3 (EAAT3), one of the glutamate transporters. Studies have suggested that disrupted EAAT3 expression resulted in neuronal death due to the excessive oxidative stress; furthermore, EAAT3 has been implicated in neurodegenerative diseases. Accordingly, our group hypothesize that autistic children may have lower detoxification capacity, and therefore are more prone to drug induced toxicity and oxidative stress. In our study, we determined if a mouse model of autism, the BTBR line, exhibits differences when compared to C57BL/6J (B6) in plasma sulfate levels, EAAT3 expression, as well as GSH levels. Plasma sulfate levels, GSH levels in plasma and brain, and EAAT3 expression in frontal cortex were measured in BTBR and B6 mice (6 weeks old, male, n=8). Our data showed that plasma sulfate levels were significantly lower in BTBR than B6 mice which have been reported. BTBR mice showed significantly lower EAAT3 expression levels in the prefrontal cortex. On the other hand, GSH and GSSG levels were similar between BTBR and B6 mice. Our findings suggest that lower plasma sulfate levels and lower EAAT3 expression in neurons may increase vulnerability to oxidative stress due to the diminished detoxification capacity and Cys availability, leading to GSH depletion in response to environmental toxins. The role of EAAT3 during development needs to be investigated in depth and BTBR mice may be a favorable animal model to study the mechanism of EAAT3 modulation. Furthermore, how altered sulfate physiology affects in normal brain development needs to be elucidated.

Disclosures: **S. Kim:** None. **M. Hilgart:** None. **A. Wadood:** None. **R. Shytle:** None.

Poster

032. Adolescent Development: Mechanisms of Vulnerability

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 32.26/A87

Topic: F.02. Animal Cognition and Behavior

Support: NIH-NIMH R01 MH082893

Title: Exercise in adolescent rats reduces renewal of extinguished instrumental behavior

Authors: *M. C. EDDY, J. T. GREEN;
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Abstract: Conditioned instrumental behaviors are voluntary actions that are controlled by their outcomes. Animals readily acquire behaviors (e.g., lever pressing) to obtain a desirable outcome (e.g., food pellet, drug delivery) and likewise learn to suppress behavior when the reinforcer is withheld. Extinction of instrumental responding is an important component of behavioral change. Extinguished behaviors can re-emerge through several associative mechanisms, including renewal. Renewal occurs when an animal is exposed to a context different from the extinction context (Bouton & Bolles, 1979), resulting in a return of extinguished responding. This return of responding demonstrates that extinction is not erasure of the original learning, but rather new inhibitory learning. Additionally, it appears that extinction learning is particularly sensitive to context (Bouton, Todd, Vurbic, & Winterbauer, 2011), a feature that presents a major challenge in treating addiction disorders clinically. Reducing renewal, therefore, is an important avenue of study. Our lab has previously demonstrated that voluntary exercise improves extradimensional set-shifting in adolescent (PD30-45) rats, which is dependent upon medial prefrontal cortex (mPFC). Because the mPFC has been suggested to underlie extinction and renewal, we asked whether exercise during adolescence (i.e., PD30-45) might affect renewal. Using a sucrose pellet reinforcer, rats with an unlocked (exercising) or locked (non-exercising) running wheel in their home cage were trained to lever press over 6 sessions in context A, and then were extinguished (lever press no longer resulted in pellet delivery) in context B. During test, rats were returned to context A (in extinction_no pellet was available; ABA renewal). No differences between groups were observed across acquisition or extinction trials. As predicted, renewal of responding occurred in non-exercising rats. Rats that had exercised also showed renewal, but responding in context A was significantly lower than non-exercising rats. Similarly, using an AAB preparation (acquisition and extinction in the same context, test in a novel context) non-exercising rats showed renewal when removed from the extinction context, while exercising rats did not show a significant increase in responding in context B. Exercise may have eliminated AAB renewal, or we may have observed a floor effect, as AAB renewal is typically of lower magnitude than ABA renewal. ABC renewal (all three

contexts novel) is currently being investigated to further explore how exercise is modifying operant renewal (and possibly mPFC function) in adolescent rats.

Disclosures: **M.C. Eddy:** None. **J.T. Green:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 33.01/A88

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NSF IOS-11212732

NIH DA034009

Title: The endocannabinoid system is altered in mice bred for high voluntary wheel running

Authors: ***Z. THOMPSON**[1], D. ARGUETA[2], J. KAUR[3], T. GARLAND, Jr.[4], N. V. DIPATRIZIO[3];
[1]Neurosci. Grad. Program, [2]Bioengineering, [3]Biomed. Sci., [4]Biol. Dept., Univ. of California, Riverside, Riverside, CA

Abstract: Endocannabinoids have many roles, including in aspects of energy balance, food reward, and voluntary locomotion. Signaling at the cannabinoid type 1 (CB1) receptor has been specifically implicated in motivation for voluntary movement, including wheel running. We studied 4 replicate lines of high-runner (HR) mice that have been bred for 75 generations based on total number of wheel revolutions on days 5 & 6 of a 6-day period of wheel access. Four replicate control (C) lines are bred without regard to wheel running. HR mice voluntarily run ~3 times as much as C, primarily by running faster. When given a CB1 receptor antagonist (SR141716; Rimonabant), HR female mice decreased their running more than C female mice, while male HR and C mice did not show a differential response. Both female and male HR mice showed a differential decrease in running (compared to C mice) when given a CB1 receptor agonist (WIN 55,212-2). We hypothesized that circulating levels of endocannabinoids (specifically, anandamide and 2-arachidonoylglycerol) differ between HR vs C mice, possibly in a sex-specific manner. 50 male and 50 female mice (half HR and half C) were allowed access to wheels for 6 days, while another 50 males and 50 females (also half HR and half C) were kept without access to wheels. Blood samples were taken during the 6th night of wheel access or no wheel access, centrifuged at 1500 g, and plasma stored at -80 C. Brains, livers, and jejunum were

also dissected. Lipids, extracted from plasma or tissue, were processed through an LCMS. Based on the previous pharmacological data, we predict higher levels of plasma endocannabinoids in the HR mice, especially in females. We also expect that mice with access to wheels for 6 days will have higher plasma levels of endocannabinoids.

Disclosures: **Z. Thompson:** None. **D. Argueta:** None. **J. Kaur:** None. **T. Garland:** None. **N.V. DiPatrizio:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 33.02/A89

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant DA027625

NIH Grant DA035316

AHA Grant 13SDG14590005

Title: GABAergic transmission and reduced modulation by endocannabinoids in adult rat rostral ventromedial medulla (RVM) neurons following persistent inflammation

Authors: *M.-H. LI, K. L. SUCHLAND, S. L. INGRAM;
Neurolog. Surgery, Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Chronic pain is a major health issue that affects ~ 20% of the population worldwide and has an estimated cost of >$500 billion a year in medical treatments and lost productivity. Recently, an *in vivo* study showed that activity of rostral ventromedial medulla (RVM) neurons changes in rats pretreated with complete Freund's adjuvant (CFA), an agent that induces persistent inflammatory pain. Using whole-cell patch-clamp recordings from *adult* rat RVM slices, we find that GABA release in the RVM is significantly increased in rats pretreated with CFA. Endocannabinoids normally inhibit presynaptic GABA release in adult RVM so that superfusion of the cannabinoid receptor 1 (CB1) selective inhibitor rimonabant (SR141716, 3 μM) increases GABAergic miniature inhibitory postsynaptic currents (mIPSCs) by 88 ± 31%. Interestingly, the ability of endocannabinoids to inhibit GABA release is reduced following CFA treatment so that SR141716 increases GABA mIPSC frequency by 13 ± 9% ($p<0.05$ compared to naive). Further, we find that the inhibition of GABA release by the CB receptor agonist Win55,212-2 is reduced in CFA-treated (11 ± 6%) compared with naïve rats (40 ± 5%; $p<0.05$).

Different from cannabinoids, opioid inhibition of GABA release was not different in naïve (52 ± 6%) compared to CFA-treated (52 ± 10%; p>0.05) RVM neurons. Increased GABA release in the RVM reduces activation of descending pain inhibitory fibers resulting in increased nociceptive transmission from primary afferent inputs to the dorsal horn of the spinal cord.

Disclosures: **M. Li:** None. **K.L. Suchland:** None. **S.L. Ingram:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 33.03/A90

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant MH085280

Title: CB1R activation enhances hippocampal excitatory neurotransmission in female adolescent rats

Authors: *C. G. REICH;
SSHS/Psychology, Ramapo Col. of New Jersey, Mahwah, NJ

Abstract: A previous study demonstrated that hippocampal CB1 receptor (CB1R) levels are lower in female adolescent animals compared to males (Reich et al., 2009). Following 21 day exposure to chronic mild stress, CB1 increased in females while decreasing in males; thus suggesting that hippocampal CB1 responds differentially to stress depending on sex. Several other lines of converging evidence clearly indicate a functional sex difference in the endocannabinoid system and behavioral reactions to exogenous cannabinoids in both human and animals (Rubino and Paralaro, 2011). However, there remains a paucity of data how these sex differences are manifested physiologically. We, therefore, begun a series of neurophysiological investigations on the endocannabinoid system in female adolescent rats. Preliminary studies show that exogenous activation of CB1 (WIN 55-212-2) enhances excitatory neurotransmission in the CA1 of female animals, while it classically decreases excitatory transmission in males. The latter is due to a CB1-mediated suppression of glutamate release. The mechanism of the observed enhancement in females is presently being investigated.

Disclosures: **C.G. Reich:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 33.04/A91

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH grant DA012413 (to D.P.)

NIH grant DA031387 (to D.P.)

Title: Peroxide-dependent sulfenylation of monoacylglycerol lipase regulates endocannabinoid signaling

Authors: *K.-M. JUNG[1], E. Y. DOTSEY[2], A. BASIT[4], D. WEI[1], J. DAGLIAN[1], F. VACONDIO[5], A. ARMIROTTI[4], M. MOR[5], D. PIOMELLI[1,4,3];
[1]Anat. & Neurobio., [2]Pharmacol., [3]Biol. Chem., Univ. of California, Irvine, Irvine, CA; [4]Drug Discovery and Develop., Inst. Italiano di Tecnologia, Genoa, Italy; [5]Pharmaceut. Dept., Univ. of Parma, Parma, Italy

Abstract: The second messenger hydrogen peroxide transduces changes in cellular redox state by reversibly oxidizing protein cysteine residues to sulfenic acid. This signaling event regulates many cellular processes, but has been never shown to occur in the brain. Here we report that hydrogen peroxide heightens endocannabinoid signaling in brain neurons through sulfenylation of cysteines C201 and C208 in monoacylglycerol lipase (MGL), a serine hydrolase that deactivates the endocannabinoid 2-arachidonoyl-sn-glycerol (2-AG) in nerve terminals. The results suggest that MGL sulfenylation may provide a presynaptic control point for 2-AG-mediated endocannabinoid signaling.

Disclosures: K. Jung: None. **E.Y. Dotsey:** None. **A. Basit:** None. **D. Wei:** None. **J. Daglian:** None. **F. Vacondio:** None. **A. Armirotti:** None. **M. Mor:** None. **D. Piomelli:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Regione Autonoma della Sardegna, Progetti di Ricerca di Base, Bando 2010

Sardinian Agency for R&TD (Sardegna Ricerche)

Title: Enhanced glutamatergic synaptic plasticity in the hippocampal CA1 field of food-restricted rats: involvement of CB1 receptors

Authors: *G. TALANI[1], V. LICHERI[2], F. BIGGIO[2], V. LOCCI[2], C. M. MOSTALLINO[1], V. MELIS[2], L. DAZZI[2], G. SITZIA[2], G. BIGGIO[2], E. SANNA[2,1];
[1]of Neuroscience, Natl. Res. Council, Monserrato, Italy; [2]Life and Envrn. Sci., Univ. of Cagliari, Monserrato, Italy

Abstract: The endogenous endocannabinoid system plays a crucial role in regulating appetite and feeding behavior in mammals as well as working memory and reward mechanisms. In order to elucidate the possible role of cannabinoid type-1 receptors (CB1Rs) in the regulation of hippocampal plasticity in animals exposed to food restriction (FR), we limited the availability of food to a 2-h daily period for 3 weeks in Sprague Dawley rats. FR rats showed a higher LTP at hippocampal CA1 excitatory synapses with a parallel increase in the probability of presynaptic glutamate release when compared to animal fed ad libitum. FR was also associated with a decreased inhibitory effect of the CB1R agonist win55,212-2 on glutamatergic fEPSPs, together with a decrease in hippocampal CB1R protein expression. In addition, hippocampal brain derived neurotrophic factor (BDNF) protein levels and mushroom dendritic spine density were significantly enhanced in FR rats. The present data underscore the important role of CB1R signaling in the regulation of glutamate release from presynaptic terminals in CA1 hippocampal field and, in turn, the increase in hippocampal excitability, long-term synaptic plasticity and dendritic spine remodeling of CA1 excitatory synapses. Our results are consistent with the hypothesis that FR in rats could have ameliorating effects on hippocampal function, and support previous published data showing increased levels of neurotrophic factors and LTP formation.
The present work has been funded by the Regione Autonoma della Sardegna, Progetti di Ricerca di Base, Bando 2010 and the Sardinian Agency for R&TD (Sardegna Ricerche).

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Poster

033. Synaptic Signaling: Retrograde Messengers

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: T32-MH065215

R01-NS078291

Title: Diacylglycerol lipase is a substrate for PKA: examining the role of dopamine and PKA in 2-arachidonoylglycerol mobilization

Authors: *B. C. SHONESY[1], J. R. STEPHENSON[1], C. R. MARKS[1], S. PATEL[2], R. J. COLBRAN[1];
[1]Mol. Physiol. and Biophysics, [2]Psychiatry, Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: The endocannabinoids (eCBs) 2-arachidonoyl glycerol (2-AG) and anandamide (AEA) are retrograde modulators of striatal synaptic transmission that have been implicated in multiple striatal-based psychiatric and neurological disorders. The identification of mechanisms regulating eCB synthesis is key to understanding the synaptic roles of eCBs in the normal and pathophysiological state. Dopamine (DA) is a crucial regulator of striatal synaptic function, and interactions between the DA and eCB signaling pathways appear to be critical in the dynamic regulation of striatal output. Although the intersection of DA and eCBs has been shown at the circuit level, the molecular mechanisms that underlie these events are still unknown. Although activation of D2-DA receptors is necessary for AEA-mediated striatal LTD, little is known about the effects of DA on 2-AG mobilization at striatal synapses. To address this, we examined the importance of D1 and D2 receptors on depolarization induced suppression of excitation (DSE) at direct and indirect pathway synapses, a form of short-term 2-AG mediated plasticity. In contrast to what is known about AEA, we found that the D1R agonist SKF-81297 enhances DSE in D1R-expressing medium spiny neurons (MSNs). Interestingly, the D1R or D2R antagonists SCH23390 and sulpiride do not significantly affect DSE in either D1R-MSNs or D2R-MSNs. 2-AG is primarily synthesized by diacylglycerol lipase alpha (DGLα) in the striatum, and we have previously shown that DGLα activity can be regulated by CaMKII phosphorylation to inhibit 2-AG synthesis (Shonesy et al., 2013. Nat Neurosci **16**:456). Therefore, since PKA is the canonical downstream target of D1 receptors, we tested whether DGLα was a substrate for PKA. These studies revealed that PKA phosphorylates DGLα at distinct sites from CaMKII. Preliminary studies indicate that PKA phosphorylation stimulates DGLα activity *in vitro*, which would be consistent with our finding that D1 receptor activation can enhance 2-AG release at striatal synapses. Moreover, initial data suggests that the enhancement of DSE by SKF-81297 in D1-MSNs can be blocked by the application of a PKA inhibitor peptide. Given the segregation of D1 and D2 receptors in direct and indirect striatal circuits, the involvement of D1 receptors in the regulation of 2-AG synthesis may be important in controlling information flowing through the striatum to modulate multiple behavioral outcomes.

Disclosures: B.C. Shonesy: None. **J.R. Stephenson:** None. **C.R. Marks:** None. **S. Patel:** None. **R.J. Colbran:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 33.07/A94

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: DA032150

Title: Far-reaching effects of fatty acid amide hydrolase knock out on the lipidome

Authors: *H. B. BRADSHAW[1], E. LEISHMAN[2], K. MACKIE[2], B. CORNETT[1];
[2]Psychological and Brain Sci., [1]Indiana Univ., Bloomington, IN

Abstract: Endogenous cannabinoids 2-arachidonoyl glycerol (2-AG), N-arachidonoyl ethanolamine (AEA), and the AEA metabolite N-arachidonoyl glycine (NAGly) are all derivatives of arachidonic acid (AA), a polyunsaturated fatty acid that is also a substrate for the production of prostaglandins (PGs). The directionality of the relationship among these lipids is an important biochemical factor in how enzymes that regulate each of the classes of lipids that potentially affects the available pools of the others. Fatty acid amide hydrolase (FAAH) is hypothesized to be responsible for the majority of the brain's AEA hydrolysis; although it can also hydrolyze other N-acyl amides. Therefore, deleting FAAH may also have effects on other bioactive lipids than AEA. This study aims to elucidate the effects of genetic deletion of FAAH on the N-acyl amide, 2-acyl glycerol, and PG lipidome in the mouse striatum, hippocampus, cerebellum, thalamus, cortex, hypothalamus, midbrain and brainstem. Six FAAH knockout (KO) mice were compared to age and sex matched wild-type mice from the same C57 genetic background. Animals were sacrificed, brains were removed and targeted areas were dissected and stored at -80C. Methanolic extracts were partially purified on C-18 solid-phase extraction columns. Eluants were analyzed with high-pressure liquid chromatography coupled to tandem mass spectrometry using an API 3000 triple quadrupole MS. Results here highlight those AA derivatives and metabolites; however, many differences were observed in other lipids. Replicating previous studies, levels of AEA were higher in FAAH KO mice across all eight brain regions. N-arachidonyl serine was likewise increased, but only in the striatum, thalamus, and brainstem. Interestingly, 2-AG levels were significantly reduced with regional specificity in the cerebellum, thalamus, and cortex of FAAH KO mice. FAAH KO mice displayed lower levels of NAGly and N-arachidonyl GABA in all brain regions, and levels of 6 additional N-arachidonyl acyl amides were significantly lowered by FAAH deletion as well. However, deleting FAAH had no impact on levels of PGE2 or free AA. These data replicate and greatly extend the finding that deletion of FAAH drives changes in a range of AA metabolites and extends this to a wider range

of N-acyl amides. This pattern of shifts in substrates and products appears largely unique to AA derivatives, demonstrating that FAAH has differential effects on lipid biosynthetic and metabolic pathways that are dependent on the specific fatty acid derivatives that are interacting with the enzyme.

Disclosures: H.B. Bradshaw: None. **E. Leishman:** None. **K. Mackie:** None. **B. Cornett:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 33.08/A95

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: DA037673

DA035068

DA009158

Title: A pro-nociceptive phenotype revealed in mice lacking fatty-acid amide hydrolase

Authors: *L. M. CAREY, IV, R. SLIVICKI, E. LEISHMAN, B. CORNETT, K. MACKIE, H. BRADSHAW, A. G. HOHMANN;
Indiana Univ., Bloomington, IN

Abstract: The endocannabinoid system represents an emerging target for pharmacotherapies aimed at treating pathological pain. Inhibition of the enzyme fatty-acid amide hydrolase (FAAH) has been proposed to enhance endocannabinoid signaling for therapeutic benefit. FAAH catalyzes metabolism of a class of biologically active lipid signaling molecules called the N-acyl amides. FAAH is the primary catabolic enzyme that degrades the endocannabinoid anandamide but it also degrades other N-acyl amides that do not bind to cannabinoid receptors. Thus, genetic deletion of FAAH is likely to produce widespread changes in the lipidome involving many bioactive lipids, in addition to anandamide. Anandamide acts as an endocannabinoid to produce antinociception at cannabinoid receptors but has also been postulated to act as an endovanilloid to produce hyperalgesia at vanilloid TRPV1 receptors. We examined the impact of genetic deletion of FAAH on pain responsiveness evoked by intradermal injection of capsaicin, the pungent ingredient in hot chili peppers. We compared capsaicin-induced mechanical allodynia, heat hyperalgesia, and nocifensive behavior in age-matched FAAH knockout (KO) and wild type

(WT) mice. Alterations in the lipidome in paw skin ipsilateral and contralateral to the site of capsaicin injection were measured using high-pressure liquid chromatography tandem mass spectrometry. Capsaicin-evoked mechanical and heat hypersensitivity and nocifensive behavior were increased in FAAH KO mice relative to WT mice. In FAAH KO mice, capsaicin-evoked hypersensitivity was blocked by the TRPV1 antagonist AMG9810. In paw skin samples derived from FAAH KO mice, profound alterations in the profiles of several lipid signaling molecules were observed ipsilateral to intradermal capsaicin injection including marked decreases in levels of 2-acyl glycerols, arachidonic acid, N-stearoyl GABA, N-acyl serines, N-acyl alanines, N-acyl ethanolamines and prostaglandin F2alpha. By contrast, in WT mice, such changes were absent; intradermal capsaicin increased levels of prostaglandin F2alpha only in paw skin samples ipsilateral to capsaicin injection. Our studies identify a previously unrecognized pro-nociceptive phenotype in FAAH KO mice challenged with capsaicin. These effects were blocked by a TRPV1 antagonist and associated with profound alterations in the lipidome that were not observed in WT animals under comparable conditions. More work is necessary to understand the mechanism underlying this pro-nociceptive phenotype if FAAH inhibition is to be employed as a therapeutic strategy for treating chronic pain in human patients.

Disclosures: **L.M. Carey:** None. **R. Slivicki:** None. **E. Leishman:** None. **B. Cornett:** None. **K. Mackie:** None. **H. Bradshaw:** None. **A.G. Hohmann:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 33.09/A96

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Opposing effects on the arachidonic acid derivative lipidome in ABHD12 and MAGL knock outs

Authors: *E. LEISHMAN, K. SPORK, A. STRAIKER, K. MACKIE, H. BRADSHAW; Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: The enzymes monoacylglycerol lipase (MAGL) and α,β-hydrolase domain 12 (ABHD12) are responsible for approximately 85% and 10% of the endogenous cannabinoid (eCB) 2-arachidonoyl glycerol's (2-AG) hydrolysis, respectively, in the rodent brain. The eCBs 2-AG, N-arachidonoyl ethanolamine (AEA), and the AEA metabolite N-arachidonoyl glycine (NAGly) are all derivatives of arachidonic acid (AA), a fatty acid (FA) that is also a substrate for the production of prostaglandins (PGs). However, other FAs undergo similar enzymatic reactions

as AA to make structural analogs (e.g. 2-linoleoyl glycerol); therefore, enzyme deletion or blockade may have broader lipidomic effects. This study aims to elucidate the effects of genetic deletion of MAGL and ABHD12 on the N-acyl amide, 2-acyl glycerol, and PG lipidome in the mouse striatum, hippocampus, cerebellum, thalamus, cortex, hypothalamus, midbrain and brainstem. 6 MAGL and 6 ABHD12 knockout (KO) mice were compared to 12 wild-type (WT) mice from the same genetic background. Animals were sacrificed, brains were removed and targeted areas were dissected and stored at -80C. Methanolic extracts were partially purified on C-18 solid-phase extraction columns. Eluants were analyzed with high pressure liquid chromatography coupled to tandem mass spectrometry using an API 3000 triple quadrupole MS. Although ABHD12 and MAGL share a common substrate, the lipidomics profiles of the KO mice drastically differ. Results here highlight AA derivatives and metabolites; though many additional classes of lipids were affected. As predicted, MAGL KO caused elevated 2-AG levels throughout the brain; however, in ABHD12 KO mice 2-AG was only elevated in the cerebellum, thalamus and midbrain. Interestingly, levels of AEA were elevated in most regions of the ABHD12 KO mice; whereas, AEA levels were lower in selected regions of the MAGL KO mice. NAGly levels followed a similar pattern to those of AEA, with region-specific increases in ABHD12 KO and decreases in MAGL KO. One of the most striking differences between the KO mice was with COX2 metabolites wherein there was a marked decrease in PGE2 and PGF2alpha in MAGL KO; conversely, there was a significant elevation in these PGs in the ABHD12 KO. Following the effects on PGs, levels of AA decreased in all regions of the MAGL KO mice, whereas, levels of AA increased in most regions of the ABHD12 KO mice. These data replicate and greatly extend the finding that deletion of MAGL drives changes in a range of AA metabolites and extends this to N-acyl amides as well as providing a novel dimension to our understanding of the roles of ABHD12.

Disclosures: **E. Leishman:** None. **K. Spork:** None. **A. Straiker:** None. **K. Mackie:** None. **H. Bradshaw:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 33.10/A97

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: National Natural Science Foundation of China No. 81271453

National Natural Science Foundation of China No. 81471125

Title: Endosomal adaptor protein APPL1, a new player in signaling from synapse to nucleus

Authors: *Y. WU, Y. YAN, X. LV, J. LUO, S. QIU;
Sch. of Med., Zhejiang Univ., Zhejiang, China

Abstract: Activity-dependent modification of gene expression is a powerful means by which the neurons builds up long-term modification. Local signaling events at synapses or axon terminals are communicated to the nucleus to elicit transcriptional responses, and thereby translates information about the external environment into internal neuronal representations. This retrograde signaling is critical to dendritic growth, synapse development, and neuronal plasticity. However, the lengths of neuronal processes pose a significant challenge for such intracellular communications. Here, we found that synaptic activity induced nuclear translocation of an endosomal adaptor protein APPL1 in the excitatory neurons. Nuclear translocation of APPL1 requires calcium influx from NMDA receptors or L-type VGCC. Futhermore,. a peptide against the NLS of APPL1 blocked synaptic activity-dependent nuclear translocation of APPL1, as well as the upregulation of CREB phosphorylation at Ser133 site in the nucleus. These data indicate that APPL1 is a key player transducing signals from synapse to nucleus and modulating gene transcription.

Disclosures: **Y. Wu:** None. **Y. Yan:** None. **X. Lv:** None. **J. Luo:** None. **S. Qiu:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 33.11/A98

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH grant NS076815.

Title: Endocannabinoids in excitatory synaptic scaling

Authors: *Y. SONG, J. ZHANG, C. CHEN;
LSUHSC, New Orleans, LA

Abstract: Synaptic scaling is a form of homeostatic plasticity, which plays an important role in stabilizing the excitability of neural circuits in response to the deprivation of excitatory or inhibitory neurotransmission. However, the mechanism of homeostasis regulation of synaptic activity is not fully understood yet. The endocannabinoid 2-arachidonoylglycerol (2-AG) functions as a retrograde signaling molecule mediating synaptic transmission and plasticity.

However, little is known about whether 2-AG signaling is involved in homeostatic regulation of miniature synaptic events at excitatory synapses in response to activity deprivation. Here, we report that chronic blockade of firing by tetrodotoxin (TTX), a sodium channel blocker, for two days resulted in increases both in the frequency and amplitude of spontaneous miniature excitatory postsynaptic currents (mEPSCs) in cultured hippocampal neurons. However, treatment with 2-AG alone or JZL184, a potent and selective inhibitor for monoacylglycerol lipase (MAGL) that hydrolyzes 2-AG, induced a CB1 receptor-dependent reduction of the frequency of mEPSCs, but not the amplitude in cultures. The TTX-increased frequency was blunted by 2-AG or JZL184 and this effect was eliminated by pharmacological or genetic inhibition of CB1 receptors. In addition, the frequency and amplitude of mEPSCs were still increased by TTX in the presence of CB1 receptor inhibition. Our results suggest that endocannabinoid signaling does not significantly contribute to the mechanism by which the strength of excitatory glutamate synapses is scaled up after chronic activity deprivation.

Disclosures: **Y. Song:** None. **J. Zhang:** None. **C. Chen:** None.

Poster

033. Synaptic Signaling: Retrograde Messengers

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 33.12/A99

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: FAPESP

CAPES

FAEPA

Title: TRPV1 and CB1 receptors in the vMPFC modulate autonomic responses in rats submitted to acute restraint stress

Authors: *T. B. MORAES NETO, A. FASINI, F. M. A. CORRÊA, L. B. M. RESSTEL; Univ. of Sao Paulo - Sch. of Med. of Ribeirao Preto, Ribeirao Preto, Brazil

Abstract: Goals: Acute restraint is an unavoidable stress situation that evokes autonomic changes, characterized by elevated mean arterial pressure (MAP), intense heart rate (HR) increases and decrease in the tail skin temperature. The ventral portion of medial prefrontal cortex (vMPFC) is a limbic structure and can modulate autonomic responses caused by stress and it can be divided in two portions, the prelimbic cortex (PL) and the infralimbic cortex (IL).

The vMPFC glutamatergic system is involved with modulation of autonomic responses evoked by restraint stress (RS). Moreover, the CB1 receptors activation reduces the local vMPFC glutamate release. Moreover, the activation of TRPV1 receptors increases the local vMPFC glutamate release. Therefore, the objective of the present work was to investigate the involvement of TRPV1 receptors and CB1 receptors in the modulation of autonomic responses evoked by RS in rats. Methods: Male Wistar rats (250-270g) had guide cannulae bilaterally implanted in the PL or IL for drug injection and a polyethylene catheter was implanted in the femoral artery for MAP and HR recording. Tail skin temperature (TST) was measured using a thermal camera. The animals were submitted to restraint, which was initiated by introducing animals into a small plastic cylindrical restraining tube (diameter =6.5cm and length =15cm) and lasted for 60 minutes. The TRPV1 antagonist 6-iodonordihydrocapsaicin (6-IODO, 3 nmol/ 200 nL) was administrate 10 minutes before the RS. The CB1 antagonist AM251 (1 pmol/ 200 nL) was administrate 5 minutes before the enzyme fatty acid amide hydrolase (FAAH) inhibitor URB597 (100 pmol/ 200 nL), that was administrate 10 minutes before the RS. Results: The RS increase both MAP ($F_{35, 350} =20.48$, $P<0.05$) and HR ($F_{35, 350} =17.44$, $P<0.05$) and decrease the TST ($F_{17, 170} =35.08$, $P<0.05$). The microinjection of 6-IODO (n=6) decrease PAM (PL: $F_{1, 350} =7.609$, $P<0.05$; IL: $F_{1, 350} =6.503$, $P<0.05$) and HR response (PL: $F_{1, 350} =16.66$, $P<0.05$; IL: $F_{1, 350} =14.59$, P0.05; IL: $P>0.05$) associate to RS when compared with vehicle treated animals (n= 6). The microinjection of AM251 and URB597 (n=6) decrease PAM ($F_{3, 700} =5.580$, $P<0.05$) and HR response ($F_{3, 700} =3.979$, $P<0.05$) and the TST drop ($F_{3, 340} =4.752$, $P<0.05$) when compared with vehicle treated animals (n= 6). Conclusion: The present results shown that vMPFC endocannabinoid system through TRPV1 receptors activation has an excitatory influence in the cardiovascular responses and the CB1 receptors activation has an excitatory influence in the cardiovascular responses and an inhibitory influence in the TST responses evoked by RS.

Disclosures: T.B. Moraes Neto: None. **A. Fasini:** None. **F.M.A. Corrêa:** None. **L.B.M. Resstel:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.01/A100

Topic: B.03. G-Protein Coupled Receptors

Support: PHS Grant RO1HD058638

PHS Grant R15DA024314

PHS Grant NS038809

Title: Estradiol rapidly attenuates ORL-1 receptor-mediated inhibition of proopiomelanocortin neurons via Gq-coupled, membrane-initiated signaling

Authors: *K. M. CONDE[1], C. MEZA[2], M. KELLY[3], K. SINCHAK[4], E. WAGNER[2];
[1]Grad. Col. of Biomed. Sci., [2]Col. of Osteo. Med. of the Pacific, Western Univ. of Hlth. Sci., Pomona, CA; [3]3 Dept. of Physiol. & Pharmacol., Oregon Hlth. and Sci. Univ., Portland, OR; [4]California State University, Long Beach, Long Beach, CA

Abstract: Ovarian estrogens act through multiple receptor signaling mechanisms that converge on hypothalamic arcuate nucleus (ARH) proopiomelanocortin (POMC) neurons. A subpopulation of these neurons project to the medial preoptic nucleus (MPN) to regulate lordosis. Orphanin FQ/nociception (OFQ/N) via its opioid-like receptor (ORL-1) regulates lordosis through direct actions on these MPN-projecting POMC neurons. Based on an ever-burgeoning precedence for fast steroid actions, we explored whether estradiol excites ARH POMC neurons by rapidly attenuating inhibitory ORL-1 signaling in these cells. Experiments were carried out in hypothalamic slices prepared from ovariectomized female rats injected one-week prior with the retrograde tracer Fluorogold into the MPN. During electrophysiologic recordings, cells were held at or near -60 mV. Post-hoc identification of neuronal phenotype was determined via immunohistofluorescence. In vehicle-treated slices OFQ/N caused a robust outward current/hyperpolarization via activation of GIRK channels. This OFQ/N-induced outward current was attenuated by 17-β estradiol (E2, 100nM). The 17α enantiomer of E2 had no effect. The OFQ/N-induced response was also attenuated by an equimolar concentration of E2 conjugated to BSA. In addition, the ability of E2 to diminish OFQ/N responsiveness was blocked by the co-administration of the estrogen receptor (ER) antagonist ICI 182,780 (1μM). The attenuating effect of E2 was mimicked by the membrane ER (mER) ligand STX (10nM) and the ERα agonist PPT (1μM), but not the GPR30 agonist G1 (3μM) or the ERβ agonist DPN (3μM). Moreover, the phospholipase C (PLC) inhibitor U73122 (20μM) restored the OFQ/N-induced outward current in the presence of E2, whereas the inactive analog U73343 (20μM) was without effect. Finally, the protein kinase C (PKC) inhibitor NPC 15437 (30μM) abrogated the estrogenic impairment of the OFQ/N-induced outward current, whereas the PKC activator PDBu (1μM) per se attenuated the OFQ/N response. These collective actions were observed in a substantial number of MPN-projecting ARH neurons positive for various markers of POMC neurons. The results reveal an ORL-1 receptor mediated inhibition of POMC neurons that is negatively modulated by estradiol. The estrogenic attenuation is stereoselective; membrane delimited, mediated via the Gq-coupled mER and ERα activation, and involves signaling through PLC and PKC. This disinhibition of POMC neurons is critical for the subsequent expression of sexual behavior in the female.

Disclosures: **K.M. Conde:** None. **C. Meza:** None. **M. Kelly:** None. **K. Sinchak:** None. **E. Wagner:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.02/A101

Topic: B.03. G-Protein Coupled Receptors

Support: Wellcome Trust Funded

Title: Inhibition of c-Src blocks morphine analgesic tolerance

Authors: ***F. BULL**[1], D. BAPTISTA-HON[1], S. KING[1], W. WALWYN[2], T. G. HALES[1]; [1]Univ. of Dundee, Dundee, United Kingdom; [2]UCLA, Los Angeles, CA

Abstract: A major limitation of morphine analgesia is the development of tolerance, which leads to the requirement for increasing doses for the treatment of persistent pain. Morphine tolerance is attenuated in βarr2-/- mice that lack β-arrestin2, which participates in mu opioid receptor (MOP) endocytosis and signaling through MAP, Akt and c-Src kinases. We previously demonstrated that βarr2-/- mice exhibit a sustained increase in MOP-mediated basal analgesia and constitutive inhibition of voltage-activated Ca^{2+} channels in DRG neurons. The latter was mimicked by inhibition of c-Src. In this study we examined whether c-Src participates in morphine analgesic tolerance and/or reinforcement. Tail withdrawal from noxious heat and conditioned place preference (CPP) were used to assess morphine analgesia and reinforcement, respectively. The reinforcing effects of morphine have been attributed to disinhibition of dopaminergic neurons of the VTA. We used the whole-cell patch-clamp technique to establish the effect of morphine on spontaneous inhibitory postsynaptic currents (sIPSCs) recorded from neurons in VTA slices. We examined several C57BL/6 strains including WT, MOP+/-, MOP-/- and βarr2-/- mice. PP2 was used to inhibit c-Src in slices, with PP3 as a control. The anti-leukaemia drug dasatinib administered ip to mice crosses the blood-brain barrier and was used to examine the behavioural effects of c-Src inhibition. Morphine caused a negligible inhibition of sIPSC frequency in VTA neurons of MOP-/- mice and was less potent in MOP+/- compared to WT neurons. A lack of βarr2 reduced morphine's inhibition of sIPSCs frequency, as did PP2, while PP3 had no effect. A lack of βarr2-/- caused a reduced locomotor response to morphine compared to WT mice, while dasatinib had no effect. βarr2-/- mice exhibited morphine (10 mg/Kg) CPP similar to WT mice. Similarly, dasatinib treated WT mice exhibited morphine CPP similar to that of controls. Morphine did not prolong MOP-/- tail withdrawal from noxious heat and had a reduced analgesic

potency in MOP+/- mice. Morphine analgesic tolerance developed faster and to a greater extent in MOP+/- mice than in WT mice. As previously reported, tolerance was reduced in βarr2-/- mice, which also exhibited basal analgesia. Dasatinib prevented morphine tolerance in WT and MOP+/- mice and caused recovery from tolerance in the latter. Our data suggest that the β-arr2/c-Src signalling system can be targeted to produce sustained morphine analgesia without affecting the drug's psychomotor effects.

Disclosures: F. Bull: None. **D. Baptista-Hon:** None. **S. King:** None. **W. Walwyn:** None. **T.G. Hales:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.03/A102

Topic: B.03. G-Protein Coupled Receptors

Support: VR

FAS

FORMAS

Title: Coherent regulation of opioid genes within and across CNS regions: interactions of co-expressing spinal circuits

Authors: *G. Y. BAKALKIN[1], M. ANDERSSON[2], V. GALATENKO[4], H. WATANABE[2], X. ZHOU[2], A. IATSYSHYNA[5], W. SUN[2], I. MITYAKINA[4], O. KONONENKO[2], I. BAZOV[3], T. YAKOVLEVA[2], D. SARKISYAN[2], N. MARKLUND[6], A. TONEVITSKY[4], D. L. ADKINS[7]; [1]Dept. of Pharmaceut. Biosciences, Uppsala Univ., Uppsala, Sweden; [2]Dept. of Pharmaceut. Biosci., Uppsala Univ., Uppsala, Sweden; [3]Uppsala Univ., Department of Pharmaceutical Biosciences, Sweden; [4]Moscow State Univ., Moscow, Russian Federation; [5]Dept. of Human Genet., Inst. of Mol. Biol. and Genet., Kyiv, Ukraine; [6]Section of Neurosurgery, Dept. of Neurosci., Uppsala Univ. Hosp., Uppsala, Sweden; [7]Med. Univ. of South Carolina Charleston, Charleston, SC

Abstract: Neuropeptides exert specific, coherent effects on formation and rewiring of neural circuits. Coordinated production of these signaling molecules and their receptors under resting conditions and upon stimulation may be a prerequisite for efficient regulation circuit's functions and connectivity. To test this hypothesis, we examined whether expression of the endogenous

opioid system (EOS) genes is co-regulated within and between three CNS areas including striatum, and dorsal and ventral cervical spinal cord. The left and right parts of these areas were analyzed separately to assess the bias associated with possible lateralization. Unilateral traumatic brain injury (CCI, controlled cortical impact centered on forelimb representation area of the motor cortex) was used as a model to impact the EOS in spinal and brain circuits, whereas unilateral sham operation (SO) was applied to control for CCI. Five groups of rats (N=10/group) including the right- and left-hemisphere CCI, right- and left-side SO rats, and naïve animals were analyzed. Analysis of variances demonstrated no significant effects of the CCI and SO on the expression of the EOS genes in the spinal cord. Interestingly, the expression was lateralized in the dorsal (Oprk1, Pdyn, Penk) and ventral (Oprd1, Oprk1, Oprm1) spinal domains. The asymmetry was also evident for the ratio of opioid receptor levels (for the Oprk1 / Oprm1 ratio in dorsal part, and for the Oprk1 / Oprm1 and Oprk1/ Oprd1 ratio in the ventral part). Left- but not right-hemisphere CCI affected Oprd1and Oprm1 expression in striatum. Analysis of the intra-area correlations identified strong, highly significant correlations between the five EOS genes in each spinal domain in both naïve group and the pooled sample of 5 groups. Right-side but not left-side surgery (each SO and CCI) reduced correlation strength in the dorsal spinal cord. Analysis of correlations between all areas / sides revealed strong highly significant positive interactions between left dorsal and left ventral spinal domains, and strong highly significant negative interactions between left dorsal and right ventral spinal domains. Network analysis confirmed these findings and identified co-regulated gene networks in the spinal cord that were specific for the left and right domains, and differentially responded to the left- and right-hemisphere CCI. The area- and side-specific co-regulation of the EOS genes may be a general principle relevant for shaping of functional connectivity within and between neural circuits in the spinal cord.

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Poster

034. Opioid and Peptide Receptors

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Brain and Behavior Research Foundation Young Investigator Award

Sidney Frank Foundation

Title: Synapse-specific persistent activation of VTA kappa opioid receptors following acute stress

Authors: *A. M. POLTER[1], R. CHEN[1], R. M. ST. LAURENT[2], J. A. KAUER[1];
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Abstract: Stressful experiences drive many adaptive and maladaptive behaviors, and even acute stressors can have lasting behavioral consequences. Emerging evidence shows that dopaminergic neurons in the ventral tegmental area (VTA) are an important locus in stress. We previously identified a long-term potentiation of GABAergic synapses onto these neurons (LTP_{GABA}) that is blocked by acute stress (Graziane et al, Neuron, 2013). Administration of a kappa opioid receptor (KOR) antagonist (norBNI) *in vivo* prevents the block of LTP_{GABA}. Intra-VTA injection of the KOR antagonist also prevents reinstatement of cocaine seeking by acute stress, suggesting that KOR-mediated regulation of VTA inhibitory plasticity may play a role in stress-induced drug seeking. Our recent work shows that a single five minute cold water swim stress blocks LTP_{GABA} for at least five days. Surprisingly, blocking KORs with norBNI even well after stress restores LTP_{GABA}, and cocaine self-administration is prevented even when norBNI is administered after stress (Polter et al, Biological Psychiatry, 2014). Here we show that the long-lasting block of LTP_{GABA} by stress is due to changes in the KOR itself. While bath application of an inverse agonist (norBNI, 100 nM) rescues LTP_{GABA} in slices from stressed animals, bath application of a neutral antagonist (6--naltrexol, 10 μM) does not (LTP magnitude: norBNI after stress=144± 18% of baseline, 6--naltrexol after stress=99±8% of baseline; $p<0.05$). These results suggest that LTP_{GABA} is blocked by constitutive activation of KORs rather than by persistently elevated dynorphin, which would be blocked by both drugs. Transient activation of KORs was sufficient to induce a lasting blockade of LTP_{GABA}, as a KOR agonist (U50488, 5 mg/kg) blocked LTP_{GABA} for 5 days (LTP: saline=140±10% of baseline, 1 day post U50488=108±5% of baseline, 5 days post U50488=99±9% of baseline). The activation of KORs by stress is synapse specific, as bath application of norBNI did not potentiate excitatory synapses on either dopaminergic (IPSC amplitude after norBNI: control=92±6% of baseline, FSS=94±2% of baseline) or GABAergic VTA neurons (IPSC amplitude after norBNI: control=109±4% of baseline, FSS=112±3% of baseline). Our results show that a single exposure to acute stress or KOR activation both cause long-lasting changes in activity of KORs specifically at GABAergic synapses in the VTA.

Disclosures: **A.M. Polter:** None. **R. Chen:** None. **R.M. St. Laurent:** None. **J.A. Kauer:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.05/A104

Topic: B.03. G-Protein Coupled Receptors

Support: NIH - NIDA/DA15014

NIH - NIDA/DA32444

Title: Variable sensitivity to morphine mediated Ferritin Heavy Chain upregulation in cortical neuronal subpopulations

Authors: *B. S. NASH[1], K. TARN[2], J. H. PITCHER[2], O. MEUCCI[2];
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Abstract: Current HIV therapies have dramatically improved patients' disease progression and quality of life, but HIV-associated neurocognitive disorder remains a prevalent and challenging problem to address in the clinic. Many HIV+ patients also abuse drugs, with opioid use being particularly common as sharing needles represents an important avenue of infection. These patients can show accelerated progression of cognitive impairment, the mechanism of which is currently under investigation. Our lab has shown that morphine and other mu-opioids upregulate Ferritin Heavy Chain (FHC) in CNS neurons, which is associated with reduction of downstream signals of the homeostatic chemokine receptor CXCR4 via its natural ligand CXCL12. This results in a host of adverse effects including reduced dendritic spine density and is correlated with enhanced cognitive decline in humans and animal models of HAND. Our experiments aim to gain insights into this novel mechanism of mu-opioid mediated CXCR4 regulation, and thereby potential future targets for novel HAND therapies. Morphine upregulates FHC in cortical neurons in a mu-opioid/g-protein dependent manner, while astrocytes seem to be unaffected *in vitro* even though they express the mu-opioid receptor. This suggests exclusivity for particular CNS cell types in their ability to upregulate FHC via opioids. FHC upregulation specifically occurs in the cytoplasm of neuronal cells as demonstrated by fractionation and confocal imaging studies, which allows it to potentially interact with CXCR4 on the cell membrane. Preliminary imaging studies show that certain neurons are more susceptible to FHC upregulation than others, and that GABA transporter-1 expressing neurons represent one of these susceptible populations. Current work is focused on exploring FHC expression after morphine treatment in inhibitory and excitatory neuronal subpopulations both *in vitro* and *in vivo*. Pilot studies suggest variability amongst neuronal subpopulations in that calretenin expressing interneurons do not upregulate

FHC after morphine, but interestingly have higher basal levels of FHC compared to calretenin negative cortical neurons. Future studies will characterize additional neuronal populations. These experiments suggest that mu-opioid usage may cause specific deficits in inhibitory neuronal circuits via FHC upregulation and subsequent CXCR4 blockade, which may induce or sustain particular features of HAND such as excitotoxicity, or other neurochemical adaptations leading to cognitive impairment.

Disclosures: **B.S. Nash:** None. **K. Tarn:** None. **J.H. Pitcher:** None. **O. Meucci:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.06/A105

Topic: B.03. G-Protein Coupled Receptors

Support: NSF Grant DGE-1252376

Title: Enkephalin-evoked catecholamine secretion in adrenal tissue

Authors: ***L. DUNAWAY**[1], L. SOMBERS[2];
[1]Chem., [2]North Carolina State Univ., Raleigh, NC

Abstract: The adrenal glands regulate physiological responses to stressors by secretion of the catecholamines, epinephrine and norepinephrine. A variety of peptides, including opioid peptides, are thought to be co-stored with the catecholamines in dense core vesicles in adrenal cells. The opioid system is strikingly complex, and the precise interaction between opioid peptides and the catecholamines has not been investigated in the adrenal gland. We have used fast-scan cyclic voltammetry coupled to carbon-fiber microelectrodes to quantify secretion of catecholamines (epinephrine and norepinephrine) in response to application of met-enkephalin, (M-ENK), and its synthetic mu opioid receptor specific analog, DAMGO, in a rat adrenal slice preparation. An acute application of M-ENK evokes catecholamine release that is sensitive to blockade by naltrexone, a non-selective opioid receptor antagonist. DPDPE, a delta opioid receptor agonist, does not elicit a physiological response. These results demonstrate that M-ENK acts in the adrenal medulla to evoke catecholamine secretion by binding to mu opioid receptors on the cell membrane, providing a specific chemical mechanism by which opioid peptides can regulate an organism's response to physiological and environmental demands.

Disclosures: **L. Dunaway:** None. **L. Sombers:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.07/A106

Topic: B.03. G-Protein Coupled Receptors

Title: A novel rabbit monoclonal antibody against the N-terminus of the rat mu-opioid receptor (MOR): selectivity for rat but not mouse MOR

Authors: *M. RAMSDEN[1], S. SCHNELL[2], M. WESSENDORF[2], A. KALYUZHNY[1]; [1]R&D Systems Inc., Minneapolis, MN; [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: In many immunofluorescent studies of the mu-opioid receptor, antisera directed against the carboxy terminal region, developed by us and others, have been successfully used to localize MOR in tissue sections. These antisera share common epitopes, are usually not species-selective and no labeling is observed with them in mice that do not express functional MOR. We have developed an antisera directed against the rat MOR amino-terminal sequence that appears not to recognize mouse MOR. We used two-color immunofluorescence to determine the spatial relationships in brain and lumbosacral spinal cord between MOR labeling obtained with the amino- and carboxyl-terminal epitopes in rats and mice. Animals were perfused with 4% formaldehyde and then sections were stained using a monoclonal rabbit anti-MOR directed at the N-terminus (R&D Systems) and a polyclonal guinea pig anti-MOR directed at the C-terminus (Arvidsson et al., 1995). These were followed by Cy2-labeled donkey anti-rabbit and Cy3-labeled donkey anti guinea pig. We observed that virtually all cell bodies, fibers and puncta were double-labeled in rat brain and spinal cord. In addition, we observed that in rats the two antibodies appeared to label identical subcellular structures. In contrast, only the C-terminal antibody stained cell bodies, fibers and puncta in tissue sections of mouse CNS. With both antibodies, staining was abolished in tissue sections where the antisera was pre-incubated with their respective immunizing peptides. The basis for this species-specificity is unclear since the amino acid sequences of the N-terminal region of rat and mouse MOR are nearly identical. These studies were supported by R&D Systems and the Department of Neuroscience, Univ. of Minnesota.

Disclosures: M. Ramsden: None. **S. Schnell:** None. **M. Wessendorf:** None. **A. Kalyuzhny:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.08/A107

Topic: B.03. G-Protein Coupled Receptors

Title: β2-AR antagonist ICI 118,551 reduces OIH after chronic opioids administration in mice

Authors: *A. SAMOSHKIN[1], O. KAMBUR[4], J. WIESKOPF[2], J. MOGIL[2], E. KALSO[5], L. DIATCHENKO[3];
[1]Dept. of Anesthesia, [2]Dept. of Psychology, [3]Dept. of Anesthesia, Dept. of Dent., McGill Univ., Montreal, QC, Canada; [4]Fac. of Med., [5]3Department of Anesthesia and Intensive Care Med. Helsinki Univ., Univ. of Helsinki, Helsinki, Finland

Abstract: Opioid analgesics are used in the treatment of moderate to severe pain, but their chronic administration of these drugs leads to unwanted adverse effects such as analgesic tolerance, dependence and opioid-induced hyperalgesia (OIH), limiting their clinical utility. A truncated 6-transmembrane (TM) isoform (lacking of the first TM and extracellular domains) of mu-opioid receptor (MOR) has been associated with high pain sensitivity and poor response to morphine. However, a newly developed potent 6TM-MOR ligand, IBNtxA, lacks the traditional adverse effects associated with classical opioids, like morphine. Moreover, recently we discovered strong evidence of 6TM-MOR coupling with β2-adrenoreceptor (AR; unpublished data). In this study we have explored these receptors interaction in reversal of OIH in mice. Chronic morphine (20-40 mg/kg) and IBNtxA (2-4 mg/kg) administered twice a day, produced hyperalgesia that manifested as sensitivity to mechanical and thermal stimuli (hot or cold). Treatment with β2-AR selective antagonist ICI 118,551 (3.25mg/kg) restored nociceptive responses to thermal stimuli back to the baseline levels. Interestingly, we also observed that ICI 118,551 produces robust synergy in analgesic efficacy upon co-administration with morphine or IBNtxA. These results further confirm the concept of β2-AR and 6TM-MOR interaction *in vivo*. We suggest that co-administration of β-blockers with opioids might be a way to achieve effective levels of analgesia while minimizing the possibility of adverse effects.

Disclosures: A. Samoshkin: None. **O. Kambur:** None. **J. Wieskopf:** None. **J. Mogil:** None. **E. Kalso:** None. **L. Diatchenko:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: B.03. G-Protein Coupled Receptors

Support: FONDECYT Nº 1110392 and 1150244

CONICYT PhD fellowship to PS

Title: CRF-BP and $CRF_{2\alpha}R$ interact primarily in the endoplasmic reticulum

Authors: P. SLATER[1], M. ANDRES[1], *K. GYSLING[2];
[1]Cell. and Mol. Biol., [2]Pontificia Univ. Catolica de Chile, Santiago, Chile

Abstract: The corticotrophin-releasing factor (CRF) family plays pivotal roles on drug addiction and stress. Intra ventral tegmental area injections of CRF, as well as stressful stimuli, induce potentiation of glutamatergic synapses onto VTA dopaminergic neurons leading to relapse to drug seeking in cocaine-experienced rats. This potentiation of glutamatergic synapses depends on CRF binding protein (CRF-BP) and type-2 CRF receptor (CRF2αR). However, the molecular mechanism underlying this functional interaction is presently unknown. We have observed that CRF-BP and CRF2αR can physically interact. We hypothesize that this interaction allows regulation of the subcellular localization of the proteins. Thus, we studied the subcellular localization of CRF-BP and CRF2αR in transfected PC12 cells, using Van Steensel co-localization analysis. The results showed that CRF-BP is localized primarily in secretion granules and CRF2αR in the endoplasmic reticulum. Interestingly, when CRF-BP and CRF2αR are co-expressed a high degree of co-localization in the endoplasmic reticulum is observed, evidencing that CRF2αR pulls CRF-BP towards the endoplasmic reticulum. This interaction and subcellular localization change are isoform-specific since it is not observed when CRF-BP is co-expressed with the CRF2βR isoform. In summary, our results show that CRF-BP and CRF2αR form a protein complex allowing modification of CRF-BP subcellular localization.

Disclosures: P. Slater: None. **M. Andres:** None. **K. Gysling:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.10/B1

Topic: B.03. G-Protein Coupled Receptors

Support: FONDECYT N° 110392 and 1150244

CONICYT PhD fellowship to HY

Title: Interaction between dopamine D1 and type-2β corticotropin releasing hormone receptors

Authors: *H. E. YARUR, M. ANDRES, K. GYSLING;
Cell. and Mol. Biol., Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract: G-protein coupled receptors (GPCR) are involved in many physiological responses and are the target of more than 50% of the drugs present in the market. Heteromerization of GPCRs is a phenomenon known as the interaction of at least two GPCRs with functional consequences distinct from individual receptors. In our laboratory, we have shown that D1 dopamine receptors (DAD1) are capable of heteromerizing with type-2α corticotropin releasing hormone receptors (CRH-R2α) when both receptors were heterogeneously co-expressed in HEK293 cells. Interestingly, CRH-R2 have three spliced variants, CRH-R2α, CRH-R2β and CRH-R2γ. CRH-R2α and CRH-R2β only differ in their N-terminal, suggesting that CRH-R2β could also interact with DAD1 Thus, we evaluated the interaction between CRH-R2β and DAD1 by co-immunoprecipitation, immunofluorescence and with the heteromer mobilization strategy (O'Dowd et al, 2005). We observed that CRH-R2β can establish an interaction with DAD1. A recombinant DAD1 with a nuclear localization sequence (DAD1-nls) is able to change the cellular localization of CRH-R2β. Our data indicate that CRH-R2β physically interacts with DAD1.

Disclosures: H.E. Yarur: None. **M. Andres:** None. **K. Gysling:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.11/B2

Topic: B.03. G-Protein Coupled Receptors

Title: *In vitro* and *in vivo* characterization of RF313, the first orally available NPFF receptor antagonist

Authors: *F. SIMONIN[1], J.-P. HUMBERT[1], I. BERTIN[1], R. QUILLET[1], V. UTARD[1], M. SCHMITT[2], J.-J. BOURGUIGNON[2], G. SIMONNET[4], E. LABOUREYRAS[4], V. ANCEL[5], V. SIMONNEAUX[5], B. BUCHER[3], T. SORG[6], H. MEZIANE[6], B. PETIT-DEMOULIÈRE[6], E. SCHNEIDER[6], B. ILIEN[1], F. BIHEL[2], K. ELHABAZI[1];

[1]Univ. De Strasbourg-CNRS, Illkirch, France; [2]Lab. d'Innovation Thérapeutique, UMR7200, [3]Lab. Biophotonique et Pharmacologie, UMR 7213, Univ. de Strasbourg - CNRS, Strasbourg - Illkirch, France; [4]UMR 5287, Univ. de Bordeaux Segalen-CNRS, Bordeaux, France; [5]Inst. des Neurosciences Cellulaires et Intégratives, CNRS, Strasbourg, France; [6]Inst. Clinique de la Souris - IGBMC, Univ. de Strasbourg-CNRS-INSERM, Strasbourg - Illkirch, France

Abstract: The mammalian neuropeptide FF (NPFF, FLFQPQRF-NH2) belongs to the family of RF-amide neuropeptides, characterized by a conserved Arg-Phe-NH2 COOH-terminal sequence. NPFF and NPFF-related peptides are the endogenous ligands of two G protein-coupled receptors named NPFF1-R (GPR147) and NPFF2-R (GPR74), and have been linked to various physiological activities including modulation of nociception and morphine analgesia. We describe here the characterization of the first orally available NPFF receptor antagonist, RF313. This compound displayed a slightly better affinity for NPFFR1 than for NPFFR2 and a potent antagonist activity at NPFFR1 receptor subtype *in vitro*. In mice, this compound had no effect by itself but prevented the hyperalgesic effect of neuropeptide VF, the endogenous agonist of NPFF1R, demonstrating its antagonist activity *in vivo*. We further showed that RF313 potentiated opiate analgesia and prevented the development of secondary hyperalgesia as well as analgesic tolerance when it was co-administered subcutaneously or orally at low doses (0.5 to 5mg/kg) in rats and mice. We also showed that this compound displayed no affinity toward the other RF-amide receptor GPR10, GPR54 and GPR103 as well as 50 different receptor targets. Moreover, RF313 displayed low toxicity when administered in mice at doses at least 10 times higher than the effective dose used to prevent the development of opioid-induced hyperalgesia and tolerance. Altogether, these data indicate that RF313 could represent a potential lead compound for the development of novel drugs that will improve opiate analgesia and reduce adverse side effects associated with chronic opiate administrations. References: Simonin F,et al., (2006) Proc. Natl. Acad. Sci. USA, 103, 466-471. Elhabazi K. et al., (2012), British J. Pharmacol. 165, 424-435.

Disclosures: **F. Simonin:** None. **J. Humbert:** None. **I. Bertin:** None. **R. Quillet:** None. **V. Utard:** None. **M. Schmitt:** None. **J. Bourguignon:** None. **G. Simonnet:** None. **E. Laboureyras:** None. **V. Ancel:** None. **V. Simonneaux:** None. **B. Bucher:** None. **T. Sorg:** None. **H. Meziane:** None. **B. Petit-Demoulière:** None. **E. Schneider:** None. **B. Ilien:** None. **F. Bihel:** None. **K. Elhabazi:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.12/B3

Topic: B.03. G-Protein Coupled Receptors

Support: JSPS KAKENHI 24890236

Title: Signaling cascades of Humanin and the derivatives through Folmyl peptide receptor like-1 (FPRL-1) : Possibility of EGFR involvement

Authors: *Y. KITA[1], N. HARADA[2], H. INUI[1,3], T. ARAKAWA[4], T. NIIKURA[5];
[1]Osaka Prefecture Univ., Osaka, Japan; [2]Applied Life Sci., Osaka Prefecture Univ., Sakai, Osaka, Japan; [3]Col. of Hlth. and Human Sci., Osaka Prefecture Univ., Habikono, Osaka, Japan; [4]Alliance Protein Labs., San Diego, CA; [5]Information and Communication Sci., Sophia Univ., Chiyoda-ku, Tokyo, Japan

Abstract: Humanin (HN), an identified cell survival factor, protects neurons from amyloid beta (Abeta) *in vitro* and reduces amyloid burden in AD model mice. To explore the functional mechanism of HN, we investigated signaling cascades activated by FPRL-1, a receptor of HN as well as cytotoxic Aβeta. FPRL-1 and the murine counterpart FPR2 are G protein-coupled receptor (GPCR) which activates extracellular signal-regulated kinase (ERK)1/2 by the ligands, Abeta, HN or Wpeptide (FPRL-1 specific agonist) etc.. The ERK1/2 phosphorylation is inhibited significantly by PTX, suggesting the involvement of Gi protein in the activation. The changes of cAMP level were also obtained upon ligand treatments. Activations of signaling molecules such as ERK, p38, JNK, and Akt were quantitatively analyzed by detecting their phosphorylation in cells expressing FPRL1 using immunoblotting. The similar levels of ERK phosphorylation were observed among the different HN derivatives, unlike with previous findings that neuroprotective activity of S14G-HN was about 1000 fold more potent than authentic HN while S7A- and C8A-HN were not neuroprotective. HN and its derivatives did not activate such signaling molecules, as p38, which were shown to be activated by FPRL-1 agonist, W peptide. Furthermore, HN seemed to activate molecules which were detected by anti-phospho EGFR antibody suggesting the so-called receptor cross-talk between FPRL-1 and EGFR. Thus, The ERK-mediated signal pathway seems not essential for the neuroprotective action of HN. These findings suggested that HN evokes signaling cascades distinct from that of natural FPRL-1 ligands, and HN activates specific signaling cascades via FPRL-1, which are relevant to HN's functions.

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Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.13/B4

Topic: B.03. G-Protein Coupled Receptors

Title: Interactions between vasoactive intestinal polypeptide and dopamine in the olfactory bulb

Authors: *K. S. KORSHUNOV[1], P. Q. TROMBLEY[2];
[2]Biol. Sci., [1]Florida State Univ., Tallahassee, FL

Abstract: The olfactory bulb (OB) receives afferent input from olfactory sensory neurons (OSNs) and uses its distinct laminar circuitry and neuronal subtypes to process odor information. The activity of the OB is circadian, and it is one of two brain regions that contains independent circadian neuronal clocks outside of the suprachiasmatic nucleus of the hypothalamus (the other is the retina, a structure in vision parallel to the OB in olfaction) (Miller et al. 2014; Ruan et al. 2008). Circadian OB activity manifests as rhythms in gene expression, neuronal activity, and odor detection. However, almost nothing is known about the cellular mechanisms that underlie the OB's circadian rhythms. A prominent cell type of the OB's circuitry is dopamine (DA) neurons found within the glomerular layer (GL) of the OB (Halasz et al. 1981; Berkowicz and Trombley 2000; Ennis et al. 2001; Davila et al. 2003). DA is implicated in sharpening odor sensation and discrimination by presynaptically inhibiting the release of glutamate from OSNs (Berkowicz and Trombley 2000; Ennis et al. 2001) and OB neurons (Davila et al., 2003). It has been shown recently that OB DA content and release varies with the time of day (Corthell et al. 2013). A second known endogenous regulator of the OB is vasoactive intestinal polypeptide (VIP), which has been implicated in regulating circadian activity within the OB (Miller et al. 2014). Unlike DA neurons, the circuitry of VIP neurons is not well established and is highly variable among mammalian species. Also, it is unknown whether VIP and DA are co-expressed by neuronal subpopulations and/or interact with each other to modulate OB activity. Hence, the goal of the present study is to test the hypothesis that subpopulations of OB neurons express DA, VIP, or both, and that VIP regulates DA neurons (as it does in the hypothalamus) and/or that DA regulates VIP neurons. To that end, we have immunohistochemical data that suggest that a subpopulation of rat DA neurons may also express VIP and VIP receptors. VIP receptors also appear to be highly expressed by neurons in the GL that are not dopaminergic. We are currently examining whether DA modulates VIP neurons and whether VIP modulates DA neurons. Such interactions may contribute to the cellular mechanism underlying circadian activity of the OB.

Disclosures: K.S. Korshunov: None. **P.Q. Trombley:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

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Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant 2R15DA021683

Title: Localization of AGRP immunoreactivity in the mouse hippocampus that expresses eGFP-tagged GHSR1a

Authors: B. GONZALEZ, *M. ISOKAWA;
Dept. of Biomedicine, Univ. Texas-Brownsville, Brownsville, TX

Abstract: The ghrelin receptor (GHSR1a) is expressed highly in the hippocampus (Mani et al., 2014) and contributes to learning and memory (Diano et al., 2006). However, in spite of intense investigation with immunohistochemical techniques and transgenic eGFP expression, what types of hippocampal neurons express GHSR1a is not well understood. Available evidence suggests hippocampal principal neurons (glutamatergic pyramidal cells and dentate granule cells) are likely the target of ghrelin (Diano et al., 2006; Cuellar and Isokawa, 2011). However, in the hypothalamus, GHSR1a is specifically localized in AgRP/NPY/GABA-containing neurons (Wang et al., 2013). Since AgRP, NPY, and GABA are all well-acknowledged neuropeptides and neurotransmitters in the hippocampus, this evidence suggests the possibility that a subset of hippocampal GABAergic interneurons that co-express AgRP and/or NPY could be the target of ghrelin. In the present study, localization of NPY and AgRP are assayed immunohistochemically in the brain of a transgenic mouse strain where GHSR1a-expressing cells are reported with eGFP. Identification of GABAergic neurons are accomplished by the immunohistochemical detection of GAD, an enzyme to synthesize GABA. Cardially-perfused brains are cryo-sectioned into 50 micrometer thick. Brain sections that contained the hippocampus and the hypothalamus (as a positive control) are collected and processed for immunohistochemistry. NPY, AgRP, GAD, and eGFP signals are visualized using a confocal microscope and the results are quantified for analysis. We observed AGRP immunoreactivity at cell soma and processes in the dentate gyrus where eGFP signals for GHSR1a were also detected. AGRP immunoreactivity was particularly dense at the inner (hilar) edge of the dentate granule cell layer, supporting our hypothesis that AGRP neurons could be the target of ghrelin in the hippocampus. We propose a novel cellular mechanism for hippocampal learning and memory that involves AGPR/GHSR1a interactions.

Disclosures: B. Gonzalez: None. **M. Isokawa:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.15/B6

Topic: B.03. G-Protein Coupled Receptors

Title: Structure based design of dual orexin receptor antagonists with a differentiated kinetic profile from suvorexant

Authors: K. A. BENNETT, S. J. AVES, J. A. CHRISTOPHER, M. CONGREVE, A. S. DORE, J. C. ERREY, J. C. PATEL, R. MOULD, B. G. TEHAN, A. ZHUKOV, F. MARSHALL, *A. BROWN;
Heptares Therapeut. Ltd, Welwyn Garden City, United Kingdom

Abstract: The orexins (orexin A and orexin B) are two neuropeptides produced by a small population of neurons in the hypothalamus which bind to G protein-coupled receptors (GPCRs) orexin-1 (OX_1) and orexin-2 (OX_2) The orexin system is a key regulator of behavioral arousal, sleep and wakefulness. There is a clear link between the orexin system and sleep modulation with loss of orexin neurones (but not receptor expression) leading to narcolepsy in humans (Tsujino N & Sakurai, 2009). Over the past decade, there has been significant drive to develop selective orexin receptor antagonists (SORAs) and dual orexin receptor antagonists (DORAs) to investigate the potential for treatment of numerous disorders including addiction and insomnia. In 2014 Merck's suvorexant (Belsomra) was approved for use in adults with insomnia. Heptares specialize in GPCR structure-based drug design. A small number of point mutations are incorporated into the receptor, increasing thermostability and creating stabilized receptors (StaR® proteins). These can be used for biophysical and structural techniques that are not usually amenable to wild-type GPCRs due to their instability when removed from the membrane. OX_1 and OX_2 StaR® proteins were purified and immobilised on biosensor chips allowing fragment screening and characterisation of receptor kinetics using surface-plasmon resonance. We identified a novel series of potent, selective, and orally efficacious dual antagonists of the orexin OX_1 and OX_2 receptors which were optimised resulting in the selection of HTL6641 as a pre-clinical candidate molecule. HTL6641 showed fast receptor kinetics (OX_1 half life ($t_{1/2}$) = 0.8 min; OX_2 $t_{1/2}$ 4.2 min) in contrast suvorexant showed significantly longer residency time (OX_1 $t_{1/2}$ = 24 min, OX_2 $t_{1/2}$ = 79 min). Fast receptor kinetics may be beneficial in reducing the potential of next day effects associated with prolonged receptor blockade, such as somnolence and driving impairment. Such observations have been noted with high doses of suvorexant (Sun et al., 2014). During the medicinal chemistry programme multiple co-crystal structures of OX_1 and OX_2 with internal series and literature compounds (such as suvorexant and SB-334867) were solved. These structures reveal, in fine detail, the binding mode of various compounds. This has

provided a detailed understanding of the similarities and differences in the ligand binding sites between the orexin receptors and is aiding the structure-based design of several series of selective OX_1 antagonists for the treatment of addiction disorders. *Sun H, Kennedy WP, Wilbraham D et al. Sleep 2013; 36: 259-67. Tsujino N & Sakurai T. Pharmacol Rev 2009; 61(2); 162-76.*

Disclosures: K.A. Bennett: A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Theapeutics Ltd. **S.J. Aves:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Theapeutics Ltd. **J.A. Christopher:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Therapeutics Ltd. **M. Congreve:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Theapeutics Ltd. **A.S. Dore:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Theapeutics Ltd. **J.C. Errey:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Theapeutics Ltd. **J.C. Patel:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Theapeutics Ltd. **R. Mould:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Theapeutics Ltd. **B.G. Tehan:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Theapeutics Ltd. **A. Zhukov:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Theapeutics Ltd. **F. Marshall:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Theapeutics Ltd. **A. Brown:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Heptares Therapeutics Ltd.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.16/B7

Topic: B.03. G-Protein Coupled Receptors

Title: Latrophilin1 adhesion GPCR signals through G protein coupling

Authors: *O. V. NAZARKO, A. KIBROM, D. ARAÇ;
Biochem. and Mol. Biol., Univ. of Chicago, Chicago, IL

Abstract: G protein-coupled receptors (GPCRs) are integral membrane proteins that play a central role in signaling pathways being key intermediaries between external stimuli and the intracellular signaling cascades and involved in regulation of nearly all processes in human body. GPCRs are essential mediators for neuronal transmission, they regulate neuronal function, modulate neuronal survival and plasticity of neuronal circuits in the nervous system and disorders in GPCR signal transduction are connected with the pathogenesis of several prominent neurodegenerative conditions including stroke, Alzheimer's, Parkinson's, and Huntington's diseases. GPCR's are today's favorite therapeutic drug targets and are studied intensively. Latrophilins (Lphn1-3) are cell-surface molecules that belong to the adhesion-type G-protein coupled receptor (GPCR) family. Lphn1 was identified as the calcium-independent receptor for α-latrotoxin, a black widow spider toxin that triggers massive neurotransmitter release from neurons and neuroendocrine cells. Mutations of Lphns have been linked to attention deficit hyperactivity disorder as well as numerous cancers. Lphns are highly expressed in the brain, and were shown to function as heterophilic cell adhesion molecules in processes such as synapse formation or maintenance. However, their signaling pathways and which effector molecules they are coupled to are not known. In our study, we demonstrated that Lphn1 signals via the inhibitory Gα (Gαi**)** G-protein. A decrease in intracellular cyclic AMP level in response to Gαi coupled signaling was shown in HEK293 cells transfected with full length LPHN1 after activation with synthetic agonist peptide. The N-terminus deletion led to constitutively more active truncated LPHN1 receptors (enhanced basal signaling) which strongly supports the idea that a tethered agonist peptide within the receptor sequence is the agonist for activation of the receptor. As an indication of Gi coupling, cAMP levels were reversed to initial level by treatment with Gi inhibitor pertussis toxin. We also designed point mutations in LPHN1 and tested their impact on constitutive or agonist induced signaling. This study could be important for further investigation of GPCR signaling modulation, which is of high significance in light of

possible treatment of different pathological conditions connected with receptor malfunctioning including neurological diseases.

Disclosures: **O.V. Nazarko:** None. **A. Kibrom:** None. **D. Araç:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.17/B8

Topic: B.03. G-Protein Coupled Receptors

Title: Conserved expression of the gpr151 receptor in habenular axonal projections of vertebrates

Authors: *B. ANTOLIN-FONTES[1], J. BROMS[2], A. TINGSTRÖM[2], I. IBAÑEZ-TALLON[1]; [1]The Rockefeller Univ., New York, NY; [2]Lund Univ., Lund, Sweden

Abstract: The habenula is a phylogenetically conserved brain structure in the epithalamus. It is a major node in the information flow between fronto-limbic brain regions and monoaminergic brainstem nuclei, thus anatomically and functionally ideally positioned to regulate emotional, motivational and cognitive behaviors. Consequently, the habenula may be critically important in the pathophysiology of psychiatric disorders such as addiction and depression. Here we investigated the expression pattern of GPR151, a G coupled-protein receptor (GPCR), whose mRNA has been identified as highly and specifically enriched in habenular neurons by *in situ* hybridization and Translating Ribosome Affinity Purification (TRAP). In the present immunohistochemical study we demonstrate a pronounced and highly specific expression of the GPR151 protein in the medial and lateral habenula of rodent brain. Specific expression was also seen in efferent habenular fibers projecting to the interpeduncular nucleus, the rostromedial tegmental area, the rhabdoid nucleus, the raphe nuclei and the dorsal tegmental nucleus. Using confocal microscopy and quantitative colocalization analysis we found that GPR151 expressing axons and terminals overlap with cholinergic, substance P-ergic and glutamatergic markers. Virtually identical expression pattern was observed in rat, mouse and zebrafish brains. Our data demonstrate that GPR151 is highly conserved, specific for a subdivision of the habenular neurocircuitry, and constitutes a promising novel target for psychiatric drug development.

Disclosures: **B. Antolin-Fontes:** None. **J. Broms:** None. **A. Tingström:** None. **I. Ibañez-Tallon:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.18/B9

Topic: B.03. G-Protein Coupled Receptors

Support: CIMO grant

RFBR 14-04-31707

RFBR 14-04-00227

RFBR 15-04-08283

Title: CGRP mediated neuroprotection against homocysteine-induced neurotoxicity in rat central and peripheral neurons

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

Abstract: The neuropeptide calcitonin gene-related peptide (CGRP), which is expressed in the central and peripheral nervous system plays a crucial pro-nociceptive role in migraine. Migraine, especially migraine with aura, is likely associated to a hyper-activation of glutamate receptors which can eventually lead to neurotoxic events. Some forms of migraine are linked to the disturbed metabolism of an endogenous amino acid: homocysteine (HCY). HCY is known a neurotoxic compound which effects are mediated by several pathways, including the activation of glutamate NMDA receptors (Abushik et al., 2014). The aim of this project was to study the role of CGRP in excitotoxicity induced by HCY in rat cultured trigeminal ganglion (TG), cerebellar and cortical neurons. D,L-HCY (100 or 500 μM) applied for 24 h induced a significant amount of cell death in all type of neurons. However, co-application of HCY together with 1 μM CGRP significantly increased the number of live TG, cerebellar and cortical neurons. A similar effect was observed with 1 μM forskolin suggesting a role for cAMP signalling in neuroprotection. Moreover, we found that CGRP signalling involves also CaMKII whereas the role of PKC was less evident. In TG neurons (rich in CGRP receptors) CGRP increased transcriptional factors Nor1 and Nur77 mRNA levels, these members of the NR4A family of orphan nuclear receptor are also known to have neuroprotective functions. Thus, the neuropeptide CGRP, apart from its pro-nociceptive role in migraine, promotes a wide ranging

protection of sensory, cerebellar and cortical neurons against the neurotoxicity induced by HCY via signalling cascades involving cAMP, CaMKII and NR4A family members.

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

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.19/B10

Topic: B.03. G-Protein Coupled Receptors

Support: MOST 104-2325-B002-010

NHRI EX103-1025NI

MOST 103-2321-B002-035

Title: Stress-induced analgesia: a sequential cascade involving neuropeptide s, orexins, substance p, glutamate and endocannabinoids

Authors: *L.-C. CHIOU[1,3], Y.-T. CHIU[2], G. CALO'[4], R. GUERRINI[5];
[2]Pharmacol., [1]Natl. Taiwan University, Med. Col., Taipei, Taiwan; [3]Grad. Inst. Brain and Mind Sci., Taipei, Taiwan; [4]Dept. of Med. Sci., [5]Dept. Chem. and Pharmaceut. Sci., Univ. of Ferrara, Ferrara, Italy

Abstract: Neuropeptide S (NPS), when given by *i.c.v.* injection, is antinociceptive in mice and activates hypothalamic orexin neurons. During stress, both NPS- and orexin-containg neurons in the hypothalamus are activated. We have previously shown that stress-induced analgesia (SIA) is mediated by orexins released from the hypothalamus, acting at the OX1R in the ventrolateral periaqueductal gray (vlPAG) to release substance P that produces disinhibition via NK1Rs through releasing glutamate that activates the $mGluR_5$-endocannabinoid(eCB)-CB1R signaling cascade. Here, we further validated a hypothesis that during stress, NPS is released to activate the orexin neurons in the hypothalamus, releasing orexins that induce analgesia through the OX1R-substance P-$mGluR_5$-eCB-CB1R sequential cascade in the vlPAG, leading to analgesia using the hot-plate test in C57BL/6 mice (male, 8-12 weeks). NPS (0.1, 0.3, 1 nmol, *i.c.v.*) significantly increased the paw withdrawal latency in mice. The antinociceptive effect of NPS (0.3 nmol) was significantly prevented by *i.c.v.* injection of [^{t}Bu-D-Gly^5]NPS (10 nmol), an NPS receptor antagonist, and by intra-vlPAG injection of the specific antagonists of NK1 (L-703,606,

0.3 nmol), OX1 (SB 334867, 15 nmol), and CB1 (AM251, 30 nmol) receptors, respectively. Importantly, a 30-min restraint stress significantly decreased the hot-plate nociceptive response in mice, and this SIA was prevented by *i.c.v.* injection of [^{t}Bu-D-Gly^{5}]NPS (10 nmol). These results suggest a novel SIA mechanism that is initiated by NPS in the hypothalamus, following by a sequential cascade in the vlPAG mediated by orexins, substance P, mGluR5 and endocannabinoids; i.e., during stress, NPS is released and activates hypothalamic orexin neurons, releasing orexins that activate the OX1R on the substance P-containing neurons in the vlPAG, releasing substance P that activates NK1 receptors on glutamatergic neurons to release massive amount of glutamate that activates perisynaptic mGluR5, generating endocannabinoids that produce retrograde inhibition of GABAergic transmission, leading to analgesia.

Disclosures: **L. Chiou:** None. **Y. Chiu:** None. **G. Calo':** None. **R. Guerrini:** None.

Poster

034. Opioid and Peptide Receptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 34.20/B11

Topic: C.17. Drugs of Abuse and Addiction

Support: The Fonds de recherche du Québec – Santé (FRQS)

Title: Delta-opioid receptor recycles from late endosomal compartments

Authors: *I. CHARFI[1,2], G. PINEYRO[1,2];
[1]Sainte-Justine Res. Ctr., Montreal, QC, Canada; [2]Univ. of Montreal, Montreal, QC, Canada

Abstract: Delta opioid receptors (DORs) are an involved in multiple brain functions and their agonists are currently considered as promising therapeutic alternatives for a variety of disorders including chronic pain, mood and learning. Substantial evidence indicates that the duration of DOR-mediated responses is very much influenced by the trafficking route these receptors follow upon activation. Classically, DORs had been considered as highly internalizing and poorly recycling receptors, but more recent evidence indicates that these trafficking patterns are also influenced by the activating ligand. Here we have characterized the post-endocytic itinerary of followed by DORs after internalization by DPDPE, an agonist that supports DOR recycling. We found that once internalized, DORs travel through compartments labeled by early-endosome antigen (EEA) and SNX1 (sorting endosomes) to accumulate in Rab9/manose-6-phospate receptor (M6PRs) positive structures, corresponding to late-endosomes and multivesicular bodies (MVBs). Interfering with transport machinery that allows retrograde recovery of M6PRs from

late endosomes to the trans-Golgi network (TGN) interfered with DOR recycling, as did blockade of TGN to membrane transport. This itinerary was confirmed both in HEK293 cells and neurons, indicating that internalized DORs can return to the membrane from late endosomal compartments where they have been classically considered as committed for degradation.

Disclosures: I. Charfi: None. **G. pineyro:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.01/B12

Topic: B.04. Ion Channels

Support: R01MH095995, FL

R01DA029091, TG

R01NS081121, TG

T32DA007287, EC

Title: Control of neuronal excitability by glycogen synthase kinase 3 in the nucleus accumbens

Authors: *M. N. NENOV[1], F. SCALA[2,1], E. CROFTON[1], Y. ZHANG[1], N. PANOVA[1], T. GREEN[1], M. D'ASCENZO[2], F. LAEZZA[1];
[1]Pharmacol. and Toxicology, Univ. of Texas Med. Br., Galveston, TX; [2]Inst. of Human Physiology, Med. School, Univ. Cattolica, Rome, Italy

Abstract: Susceptibility to psychiatric disorders is associated with gene and environment interactions that can predispose or protect individuals against these brain pathologies. Enriched environmental conditions (EC) can exert protective effects opposing maladaptive neuronal plasticity of the brain circuit that underlies these aberrant behaviors. Using unbiased transcriptomic analysis in the nucleus accumbens (NAc) of EC rats mRNAs coding for glycogen synthase kinase 3 (GSK3) and the voltage-gated Na+ channel Nav1.6 were identified as part of a protective genetic program against addiction and psychiatric disorders. Using *in vivo* Adeno-Associated Virus (AAV) vector expression for selective genetic silencing and acute brain slice patch-clamp electrophysiology, we found that silencing of either GSK3 or Nav1.6 with selective AAV short hairpins leads to a reduction in maximum firing frequency and increased in the action

potential threshold. Accordingly, we show that application of GSK3 inhibitor CHIR99021 in medium spiny neurons in both the NAc core and shell significantly suppresses maximum firing frequency and reduces Na+ persistent currents compared to vehicle treated cells. In complementary studies in HEK293 cells stably expressing Nav1.6 channels, we show that either CHIR99021 or GSK3 inhibitor XIII induce a significant reduction of Na+ peak current density compared to control along with significant changes in the voltage-dependence of the channel activation and/or steady-state inactivation that are inhibitor-specific. Based on this evidence, we propose that changes in GSK3 and Nav1.6 expression might coordinate rewiring of the NAc with effects on depression-, anxiety- and addiction-related behaviors.

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Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.02/B13

Topic: B.04. Ion Channels

Support: VA merit grant

CT stem cell research program

Kenneth Rainin Foundation

Title: Multi-electrode array (MEA) analysis of disease-causing Nav1.7 A1632G mutation in inherited erythromelalgia, a chronic pain syndrome

Authors: *Y. YANG[1], J. HUANG[2], M. ESTACION[3], D. B. HORTON[4], S. D. DIB-HAJJ[3], S. G. WAXMAN[3];
[1]Neurol., VA CT Healthcare Syst., West Haven, CT; [2]VA healthercare, west haven, CT; [3]VA healthercare, West Haven, CT; [4]Nemours A.I. duPont Hosp. for Children, Wilmington, DE

Abstract: Voltage-gated sodium channel Nav1.7 is a central player in human pain. Mutations in Nav1.7 produce several human pain syndromes including inherited erythromelalgia (IEM), a syndrome in which patients suffer from episodes of intense burning pain that are often triggered by warmth. Patch-clamp studies show that gain-of-function Nav1.7 mutations from patients with IEM increase DRG neuron excitability. However, the long-term effects of expression of Nav1.7

mutations in cultured DRG neurons are not known, and the response of DRG neurons expressing mutant Nav1.7 channels to thermal stimuli has not been well-documented. Multielectrode arrays (MEA) provide a non-invasive extracellular recording approach, typically having ~64 micro-electrodes engineered on the bottom of a culture dish, which can continuously record action potentials from a population of neurons over a period of up to several weeks. Using MEA and traditional patch-clamp, we have studied a newly-identified Nav1.7 mutation, A1632G, from a patient with IEM. This mutation shifts fast-inactivation of the channel in a depolarized direction and significantly increases spontaneous firing in a population of transfected DRG neurons. We are studying the effect of physiologically relevant variations of temperature on the firing of these neurons. Through MEA and traditional electrophysiologic approaches, we have better characterized the pathophysiology of a novel disease-causing Nav1.7 mutation expressed in DRG neurons.

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Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.03/B14

Topic: B.04. Ion Channels

Support: R01 MH095995 (FL)

R21 MH093844 (JZ)

NIGMS Grant No.1 T32 GM089657-04 (SRA)

Title: Identification of new druggable pockets at the FGF14: Voltage-gated sodium channel 1.6 complex

Authors: ***S. R. ALI**, Z. LIU, A. K. SINGH, M. N. NENOV, J. ZHOU, F. LAEZZA;
Dept. of Pharmacol. & Toxicology, Univ. of Texas Med. Br., Galveston, TX

Abstract: Voltage-gated sodium (Nav) channels are responsible for the initiation and propagation of transient depolarizing currents that control neuronal excitability. Dysregulation of specific Nav channel isoforms is found across a wide range of brain disorders associated with motor and cognitive disabilities. Unfortunately, currently available drugs targeting Nav channels

are directed against highly conserved domains of the protein and as such lack isoform specificity. The macromolecular complex of Nav channels is a source of less conserved protein-protein interaction (PPI) interfaces that represent a unique opportunity for the design of isoform-specific chemical leads against Nav channelopathies. The intracellular fibroblast growth factor 14, FGF14, is a biologically relevant component of the neuronal Nav channel complex controlling gating, stability and trafficking of native Nav channels. Through a monomeric interaction with the intracellular C-terminal tail of Nav channel α subunits, FGF14 binds and modulates the activity of Nav channels in an isoform-specific manner with potency and affinity unique to any other identified interactors. Here, we have reconstituted the FGF14:Nav1.6 complex in live cells using the split-luciferase complementation assay (LCA) and through site-direct mutagenesis identified "hot-spots" at the FGF14 surface critical for binding to Nav1.6. By patch-clamp electrophysiology, we have further identified that Y158 and V160 located in the β8/9 of FGF14 are required to modulate Nav1.6-encoded current. Subsequently, we have designed short peptide fragments that targeted of β12-strand and β8-β9 loop of FGF14 and validated their in-cell activity as inhibitors of the FGF14:Nav1.6 complex. These breakthrough results identify the FGF14 β8-β9 as part of potential druggable pocket against the FGF14:Nav1.6 complex. Small molecules including short peptides and peptidomimetics targeting this pocket might give rise to a new class of unconventional PPI-based allosteric modulators of Nav channels that could restore malfunction of neuronal excitability and plasticity. These results provide fundamental new knowledge for the design of new leads targeting the Nav channel macromolecular complex. We expect our studies to have a broad impact in the drug design against a wide range of still untreatable brain disorders. Funding: Supported by PhRMA Foundation (FL) NIH R01 MH095995 (FL), R21 MH093844 (JZ), and Gulf Coast Consortia NIGMS Grant No.1 T32 GM089657-04 (SRA).

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Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.04/B15

Topic: B.04. Ion Channels

Support: NIH Grant HL074011

NIH Grant HL108609

Title: The background sodium channel NALCN regulates basal excitability of chemosensitive retrotrapezoid nucleus neurons and stimulation of breathing by CO2

Authors: *Y. SHI, C. ABE, B. HOLLOWAY, S. SHU, E. PEREZ-REYES, R. STORNETTA, P. GUYENET, D. BAYLISS;
Univ. of Virginia, Charlottesville, VA

Abstract: CO2/H+-sensitive neurons of the retrotrapezoid nucleus (RTN) are a nexus for integration of multiple respiratory-related inputs in service of homeostatic regulation of the rate and depth of breathing, blood gases, and arterial pH. The spike firing activity of RTN neurons controls respiratory output. Using a transgenic mouse line in which RTN chemosensory neurons are marked with GFP, we identified two populations of RTN neurons -- Type I and Type II -- with different firing activities evident over distinct pH ranges. The higher baseline firing rate in Type II cells is associated with greater inward current at -60 mV and larger NMDG-sensitive, TTX-resistant current. We hypothesized that NALCN, a “leak” Na+ channel might underlie this RTN current. Single cell RT-PCR (scPCR) and *in situ* hybridization showed that NALCN is expressed in nearly all RTN neurons (>90%), and quantitative scPCR revealed that NALCN expression is highest in Type II cells. Using viral-mediated shRNA knockdown of NALCN, we found that NALCN depletion favors Type I properties *in vitro* and blunts ventilatory responses to CO2 *in vivo*. Therefore, NALCN plays an important role in establishing baseline excitability of RTN neurons and contributes to control of breathing by these cells.

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Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.05/B16

Topic: B.04. Ion Channels

Support: NIH Grant R01NS053422

NIH Diversity Supplement NS053422

NIH Grant F31NS090837

Title: FHF2A negatively regulates resurgent currents in sensory neurons

Authors: *C. M. BARBOSA NUNEZ, T. R. CUMMINS;
Dept. of Pharmacol. and Toxicology, Indiana Univ., Indianapolis, IN

Abstract: Resurgent sodium currents (INaRs) are an atypical sodium current that is mediated by an alternate mechanism of inactivation termed open channel block. Increased INaRs in sensory neurons have been implicated in different pain pathologies. However, our ability to selectively target INaRs is hindered by our lack of knowledge on how INaRs are modulated. In this study we investigated the potential regulation of INaRs in sensory neurons by Fibroblast Growth Factor Homologous Factor 2A (FHF2A). FHF2A variant contains a long N-terminus sequence that can interact with sodium channels and induce long-term inactivation. Previous studies with Navβ4 derived peptide (open channel blocker) and FHF2A derived peptide (long term inactivation particle) suggest that these factors compete for the open channel state. Therefore, we hypothesized that FHF2A limits sensory neurons capacity to generate resurgent current by outcompeting the open channel blocker. To test this hypothesis we overexpressed FHF2A or chimeric constructs of FHF2A and Navβ4. The FHF2A-(β4) chimera was generated by substituting the long term inactivation particle sequence (F2A) with the open channel particle sequence (β4) and vice-versa for the Navβ4-(F2A) chimera. Overexpression experiments were performed using recombinant expression in primary cultured DRG neurons. Nav1.6r was biolistically transfected with tagged constructs of FHF2A, FHF2A-(β4), Navβ4-(F2A) or vector (tag only). Endogenous currents were eliminated and whole-cell patch-clamp recording were obtained. Overexpression of the FHF2A decreased INaR positive neurons and INaR amplitude relative to control. FHF2A overexpression increased the fraction of channels in long-term inactivated states and slowed channel recovery relative to control. In contrast, overexpression of FHF2A-(β4) chimera increased resurgent current amplitude and INaR positive neurons. Recovery from inactivation was enhanced in FHF2A-(β4) group relative to control. The fraction of channels in long-term inactivated states was not different between FHF2A-(β4) and control. Overexpression of Navβ4-(F2A) abolished resurgent current generation. However, the fraction of channels in long-term inactivated states was not different than control suggesting that inhibition of INaR observed in this group may be due to a dominant negative effect of an inactive Navβ4. Overall, our results suggest that FHF2A negatively regulates INaRs in sensory neurons by mediating long-term inactivation. Interestingly, Navβ4-(F2A) data supports that NavB4 open channel blocker sequence is important for mediating INaR.

Disclosures: C.M. Barbosa Nunez: None. **T.R. Cummins:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

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Program#/Poster#: 35.06/B17

Topic: B.04. Ion Channels

Support: U01-MH-098953

T32-NS043126

Title: Electrophysiological phenotyping of human cortical neurons in slices and dissociated culture

Authors: *A. ULYANOVA[1], J.-H. LEE[2], S. BREM[1], T. LUCAS[1], D. M. O'ROURKE[1], M. GROVOLA[1], J. WANG[2], Y. NA[3], J. SINGH[2], D. H. SMITH[1], J. KIM[3], J. EBERWINE[2], J.-Y. SUL[2], M. S. GRADY[1], J. A. WOLF[1,4];
[1]Neurosurg., [2]Pharmacol., [3]Biol., Univ. of Pennsylvania, Philadelphia, PA; [4]Philadelphia Veterans Affairs Med. Ctr., Philadelphia, PA

Abstract: The electrophysiological and morphological phenotyping of human neurons was performed on resected cortical and hippocampal tissue from cases of Communicating Hydrocephalus, Epilepsy, Normal Pressure Hydrocephalus as well as primary and secondary brain tumors. The differences in electrophysiological properties of adult human neurons are being correlated to the single-cell analysis of the mRNA transcriptome. With IRB approval 79 patients were enrolled with informed consent. For each enrolled patient, a case report form was populated with the subject information, which includes cortical location of the tissue as well as past medical history, all current medications and pathology reports (25-86 years old). Brain tissue that would otherwise be discarded was collected into ice-cold oxygenated artificial cerebrospinal fluid to preserve the necessary cellular and cortical circuitry components. Spontaneous and induced action potentials (APs) as well as concurrent field potentials were recorded from 350um thick cortical and hippocampal slices using intracellular sharp electrodes. Dissociated adult human neurons survived in culture for up to 6 months. Using whole-cell patch-clamp technique, ionic currents and changes in membrane potentials were analyzed. Resting membrane potential, amplitude of currents through voltage-gated channels, as well as consequent APs generation was monitored over time (days) in culture. The associated differences in electrophysiological properties of adult human neurons will be correlated to the single-cell transcriptome throughout various stages of culture. In order to develop a better understanding of the variability of the mRNA profile of individual cells, as well as in various disease states, identified correlations will be compared among different patient diagnoses.

Disclosures: **A. Ulyanova:** None. **J. Lee:** None. **S. Brem:** None. **T. Lucas:** None. **D.M. O'Rourke:** None. **M. Grovola:** None. **J. Wang:** None. **Y. Na:** None. **J. Singh:** None. **D.H.**

Smith: None. **J. Kim:** None. **J. Eberwine:** None. **J. Sul:** None. **M.S. Grady:** None. **J.A. Wolf:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.07/B18

Topic: B.04. Ion Channels

Support: R01MH095995, FL

T32ES007254, TJ

Title: Triciribine potentiates Nav1.6-encoded currents and increases excitability of medium spiny neurons in the nucleus accumbens

Authors: *F. SCALA[1,2], M. N. NENOV[2], M. A. ALSHAMMARI[2], T. F. JAMES[2], N. I. PANOVA[2], C. GRASSI[1], M. D'ASCENZO[1], F. LAEZZA[2];
[1]Univ. Cattolica Del Sacro Cuore, Rome, Italy; [2]Univ. of Texas Med. Br., Galveston, TX

Abstract: Evidence indicates that dysfunction of the Akt/protein kinase B pathway correlates with the phenotypic manifestation of psychiatric disorders and neurodegenerative diseases. Yet, the molecular endpoints of this pathway in neurons remain poorly understood. Recent studies from our group identified glycogen synthase kinase 3 (GSK3), a well characterized downstream target of AKT as a modulator of the voltage-gated Na+ (Nav) channels. Here, we posited that inhibition of AKT might exert effects on Nav channel function and excitability in neurons. Using whole-cell patch-clamp recordings in HEK293 cells stably expressing Nav1.6 channels we show that pharmacological inhibition of AKT (triciribine 20 uM) induces a significant potentiation of Nav1.6 peak current density with no changes in the channel biophysical properties. Accordingly, prolonged application of triciribine (12-24 h) in mature primary hippocampal neurons leads to increased firing frequency and decreased action potential threshold. We then examined the effect of triciribine in acute brain slices from the nucleus accumbens (NAc), a brain area associated with high expression of Nav1.6. Whole-cell patch clamp recordings from medium spiny neurons revealed that triciribine increases repetitive firing, decreases action potential threshold and potentiates persistent Na+ current, a functional signature of Nav1.6 channels. Taken together, these findings provide evidence for a molecular target of the AKT pathway that effect on Nav1.6

channels. Thus, suppression of the AKT pathway might promote a hyper excitable state that could contribute to neuronal circuit dysfunction associated with brain disorders.

Disclosures: **F. Scala:** None. **M.N. Nenov:** None. **M.A. Alshammari:** None. **T.F. James:** None. **N.I. Panova:** None. **C. Grassi:** None. **M. D'Ascenzo:** None. **F. Laezza:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.08/B19

Topic: B.04. Ion Channels

Support: BMBF 01GQ1005A

BMBF 01GQ1005B

BMBF 01GQ1005D

CNMPB A1-A6

Title: What is the high sodium channel density in the axon initial segment good for?

Authors: ***A. NEEF**[1,2], E. LAZAROV[1,3], M. DANNEMEYER[4], M. J. GUTNICK[3], F. WOLF[1,2]; [1]MPI For Dynamics and Self-Organization, Goettingen, Germany; [2]Bernstein Ctr. for Computat. Neurosci., Goettingen, Germany; [3]Koret Sch. of Vet. Med., Hebrew Univ., Jerusalem, Israel; [4]Georg August Univ., Goettingen, Germany

Abstract: In many types of neurons, the density of sodium channels is higher in the axon initial segment (AIS) as compared to the soma. The AIS is also the place of action potential (AP) initiation and the high local density of sodium channels is often seen as a crucial requirement for axonal AP initiation. On the other hand, recent theoretical [1] and experimental[2] studies suggest that other parameters, such as the electrotonic distance from the soma, contribute significantly to the excitability of an axonal segment. We studied AP initiation in cultured hippocampal neurons at different stages of maturation with patch clamp and by immuno-histochemistry and fluorescence microscopy to visualize molecular components of the AIS. For voltage gated sodium channels a pan-antibody, recognizing all neuronal subtypes, was used. Beginning at 7 days *in vitro* (DIV), we recorded somatic AP waveforms. They had a biphasic shape due to non-local drive by lateral current that preceded local, somatic sodium channel

activation. This indicates that APs were initiated in the axon. Over the next 3 weeks, the channel densities in soma and axon increased and the somatic AP shape matured to a form tat closely resembled APs recorded from hippocampal neurons in tissue slices. In neurons derived from mice with the qv3J mutation of beta-IV-spectrin [3], we observed that the sodium channel immuno-staining in the AIS failed to increase while somatic and dendritic channel densities developed normally. Consistent with a reduced axonal sodium channel density, the amount of lateral current that drove early somatic depolarization was decreased in the mutants, while the somatic voltage threshold for AP initiation was increased. At DIV 21 to 28, the sodium channel immuno-staining in the axon was often as weak as the somatic staining, however, AP initiation still occurred in the axon. Preliminary data suggests, that part of the remaining axonal sodium channels are of the subtype NaV1.6, featuring a slightly lower activation threshold. Beyond DIV 21, an increasing fraction of the neurons displayed monophasic AP-shapes consistent with somatic AP initiation. Additional experiments are now performed to determine the initiation site in those cases. We conclude that high axonal sodium channel densities are not required to shift the action potential generation into the soma. Numerical simulations of multi-compartment neuron models using realistic sodium channel densities and properties support the notion, that axonal AP initiation can be achieved without a high density ration between soma and axon. [1] Brette 2013 [2] Baranauskas et al. 2013 [3] Lacas-Gervais et al. 2004

Disclosures: **A. Neef:** None. **E. Lazarov:** None. **M. Dannemeyer:** None. **M.J. Gutnick:** None. **F. Wolf:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.09/B20

Topic: B.04. Ion Channels

Support: Grant-in-Aid for Scientific Research(C)15K08871

Title: The effect of NKCC1 on aquaporin-4 induced astrocyte swelling

Authors: *R. KATADA, K. SUGIMOTO, K. NAKAMA, H. YOSHIZAWA, H. MATSUMOTO;
Osaka Univ. Fac. of Med., Suita, Japan

Abstract: Brain edema is the cause of poor prognosis after traumatic brain injury (TBI). Aquaporin-4 (AQP4), an water channel, is related to brain edema formation after TBI under ethanol consumption (Katada et al, Am J Pathol 2012). Sodium ion concentration in blood is decreased after TBI in rat under ethanol exposure. In fresh water drowning case (hyponatremia), brain AQP4 expression is increased, on the other hand, it is decreased in salt water drowning case (hypernatremia). AQP4 is regulated by or co-expressed with sodium channel, Na(+)-K(+)-2Cl(-) co-transporter-1 (NKCC1). From these findings, we hypothesized that the interaction between AQP4 and sodium channel may affect AQP4 expression change by sodium ion. In the present study, rat primary astrocytes were obtained from rat pup cortices. Astrocytes were incubated in iso-sodium MEM (NaCl: 680 mg/dl), hypo-sodium (NaCl: 410 mg/dl) or hyper-sodium MEM medium (NaCl: 950 mg/dl) with 10% calf serum in 2 hr with or without ethanol. NKCC1 siRNA was used for checking whether NKCC1 expression would affect AQP4 expression or not. Astrocyte AQP4 and alpha-syntrophin, an AQP4 anchor protein, expressions were checked by western blotting and immunohistochemistry. And immunoprecipitation for AQP4 and NKCC1 in cytosol or membrane extracts was performed. AQP4 expression was increased in 3 hr hypo-sodium medium exposure. However, Hyper-sodium did not change AQP4 expression significantly. NKCC1 knockdown decreased AQP4 expression under hypo-sodium condition. Alpha-syntrophin was increased by AQP4 knockdown under ethanol and hyper-sodium condition. The binding activity for AQP4 and NKCC1 was changed by each extract. These findings suggest that brain edema increasing by AQP4 and sodium ion was involved in the interaction between AQP4 and NKCC1.

Disclosures: R. Katada: None. **K. Sugimoto:** None. **K. Nakama:** None. **H. Yoshizawa:** None. **H. Matsumoto:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.10/B21

Topic: B.04. Ion Channels

Support: European Union Seventh Framework Programme FP7/2007-2013, 602273

Rehabilitation Research Service and Medical Research Service, Department of Veterans Affairs

Center for Neuroscience & Regeneration Research is a Collaboration of the Paralyzed Veterans of America with Yale University

Title: Gain-of-function Nav1.9 F1689L mutation in idiopathic small nerve fiber neuropathy

Authors: *B. S. TANAKA[1,2], M. ESTACION[1,2], J. G. J. HOEIJMAKERS[3], M. M. GERRITS[4], G. LAURIA[5], I. S. J. MERKIES[3,6], C. G. FABER[3], S. DIB-HAJJ[1,2], S. G. WAXMAN[1,2]; [1]Dept. of Neurol., Yale Univ. Sch. of Med., New Haven, CT; [2]Ctr. for Neurosci. and Regeneration Res., Veterans Affairs Med. Ctr., West Haven, CT; [3]Neurol., [4]Clin. Genomics, Univ. Med. Ctr. Maastricht, Maastricht, Netherlands; [5]Neuroalgology, IRCCS Fndn. Carlo Besta, Milan, Italy; [6]Neurol., Spaarne Hosp., Hoofddorp, Netherlands

Abstract: Painful peripheral neuropathy often occurs without identifiable causes, but no systematic genetic studies have been performed in patients with this disorder. We sought to identify a genetic basis for painful peripheral neuropathy by screening these patients for mutations in the SCN9A, SCN10A and SCN11A genes, encoding voltage-gated sodium channels Nav1.7, Nav1.8, and Nav1.9 which are preferentially expressed in small-diameter peripheral sensory neurons. Patients referred with painful neuropathy and no underlying etiology for SFN, were screened for mutations in SCN9A, SCN10A, and SCN11A and functional analyses were carried out. Here we report the identification of a new mutation in SCN11A, and no mutations in SCN9A or SCN10A, in an individual with painful peripheral neuropathy. This variant encodes a missense substitution in SCN11A resulting in a change of the Phenylalanine at position 1689 to Leucine (F1689L) in the C-terminus of human Nav1.9. Functional analysis using current-clamp revealed gain-of-function changes at the neuronal level. The effect of the mutation on the channel properties are being evaluated by voltage-clamp recordings. These findings provide new evidence supporting the contribution of Nav1.9 to pain associated with painful neuropathy, and widens the spectrum of gain-of-function mutations in Nav1.9 to include substitutions within the C-terminus of the channel.

Disclosures: B.S. Tanaka: None. **M. Estacion:** None. **J.G.J. Hoeijmakers:** None. **M.M. Gerrits:** None. **G. Lauria:** None. **I.S.J. Merkies:** None. **C.G. Faber:** None. **S. Dib-Hajj:** None. **S.G. Waxman:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.11/B22

Topic: B.04. Ion Channels

Title: Sodium channel accessory β4 subunits control long-term depression in nucleus accumbens medium spiny neurons

Authors: *S. SAHA, X. JI, G. E. MARTIN;
Psychiatry, Brudnick Neuropsychiatric Res. Inst., Worcester, MA

Abstract: Voltage-gated sodium channels are essential for generating the initial rapid depolarization of neuronal membrane potential known as action potentials that enable cell-to-cell communication, propagation of signals throughout the brain, and control the induction of synaptic plasticity. Although all brain neurons express one or several variants coding for the core pore-forming α subunit, the expression of the β1 to β4 auxiliary subunit varies greatly. Of particular interest is the β4 subunit, also called Scn4b, whose expression in the dorsal and ventral (i.e. nucleus accumbens - NAc) striatum is much higher compared to other brain regions. Scn4b confers sodium channels unique gating properties, yet its role on neuronal activity and synaptic plasticity remains poorly understood. Combining whole-cell patch-clamp recordings and two-photon calcium imaging in Scn4b knockout and knockdown mice, we found that down regulation of Scn4b altered the properties of action potentials (APs) in core accumbens medium spiny neurons (MSNs). These alterations resulted in a strong reduction of spike-timing-dependent long-term depression (tLTD) in MSNs. In contrast, long-term potentiation (tLTP) remained unaffected. Finally, it reduced the ability of backpropagating action potentials to evoke calcium signals in dendrites of MSNs. Taken together, these data indicate that the Scn4b subunit selectively controls tLTD by modulating dendritic calcium transients evoked by backpropagating action potentials.

Disclosures: **S. Saha:** None. **X. Ji:** None. **G.E. Martin:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.12/B23

Topic: B.04. Ion Channels

Support: NIH Grant R01 NS053792

NIH Grant T32 NS007491

NIH Grant F31 NS077700

Title: Evaluation of Cacna1g as a candidate modifier of epilepsy in the Scn2aQ54 mouse model

Authors: *J. CALHOUN, N. A. HAWKINS, N. J. ZACHWIEJA, J. A. KEARNEY; Northwestern Univ., Chicago, IL

Abstract: Epilepsy is a common neurological disorder characterized by recurrent seizures. Mutations in genes encoding voltage-gated sodium channels are responsible for several types of human epilepsy. More than 1000 mutations in the neuronal sodium channels *SCN1A* and *SCN2A* have been identified in human patients with several epilepsy syndromes, including Genetic Epilepsy with Febrile Seizures Plus, Dravet Syndrome, and Benign Familial Neonatal-Infantile Seizures. A common feature of genetic epilepsies is variable expressivity in family members with the same mutation. We generated mouse models with mutations in voltage-gated sodium channels *Scn1a* and *Scn2a* that cause epilepsy phenotypes with different underlying mechanisms. Both mouse models show strain-dependent differences in phenotype severity. These observations demonstrate that genetic modifiers act to influence the expressivity of epilepsy. *Cacna1g*, encoding the Cav3.1 voltage-gated calcium channel subunit, was identified as a candidate modifier of epilepsy in the $Scn2a^{Q54}$ mouse model using genetic mapping and RNA-seq transcriptome analysis. In this study, transgenic mice were generated with increased expression of *Cacna1g* in order to test the hypothesis that elevated expression of *Cacna1g* will increase the severity of the epilepsy phenotype in the $Scn2a^{Q54}$ mouse model. We observed increased seizure frequency in $Scn2a^{Q54}$ mice with elevated *Cacna1g* expression relative to wildtype littermates. This demonstrates that *Cacna1g* is a modifier of the $Scn2a^{Q54}$ epilepsy phenotype and suggests that *Cacna1g* may be a potential therapeutic target for epilepsy. The identification of genetic modifiers that influence susceptibility and disease progression will provide insight into the etiology of epilepsy and suggest novel therapies for improved treatment of human patients.

Disclosures: J. Calhoun: None. **N.A. Hawkins:** None. **N.J. Zachwieja:** None. **J.A. Kearney:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.13/B24

Topic: B.04. Ion Channels

Support: Rehabilitation Research Service and Medical Research Service, Department of Veterans Affairs

#602273 European Union Seventh Framework Programme FP7/2007-2013

Paralyzed Veterans of America

Title: Gain-of-function mutation in voltage-sensing domain of $Na_v1.8$ associated with idiopathic small fiber neuropathy

Authors: *J. HUANG[1], P. ZHAO[1], J. G. J. HOEIJMAKERS[2], M. M. GERRITS[3], G. P. LAURIA[4], C. G. FABER[2], I. S. J. MERKIES[2,5], S. D. DIB-HAJJ[1], S. G. WAXMAN[1]; [1]Dept. of Neurol. and Ctr. for Neurosci. and Regeneration Res., Yale Univ. Sch. of Medicine/Veterans Affairs Med. Ctr., West Haven, CT; [2]Neurol., [3]Clin. Genet., Univ. Med. Ctr. Maastricht, Maastricht, Netherlands; [4]Headache and Neuroalgology Unit, Fondazione IRCCS Inst. Neurologico 'Carlo Besta', Milan, Italy; [5]Neurol., Spaarne Hosp., Hoofddorp, Netherlands

Abstract: Variants of sodium channel $Na_v1.8$, a critical molecule for repetitive firing in dorsal root ganglion (DRG) neurons, were previously identified in patients diagnosed with idiopathic painful small fiber neuropathy (I-SFN), providing an association with human pain disorders. Four of these $Na_v1.8$ variants (L554P, A1304T, I1706V, and G1662S) have been shown to manifest gain-of-function attributes that render DRG neurons hyperexcitable. These mutants alter biophysical properties of the $Na_v1.8$ channel, including hyperpolarizing activation, depolarizing fast-inactivation, facilitating recovery from fast-inactivation and enhancing ramp current. We have now evaluated additional I-SFN patients and report a novel *SCN10A* variant occurring in a voltage-sensing domain of the channel. Current-clamp studies in adult rat small DRG neurons (< 30 μm) revealed that this mutation significantly increases action potential-firing frequency and the population of repetitively firing neurons as compared to wild-type channel. The percentage of spontaneous firing neurons did not differ between neurons expressing wild-type or mutant channels. Mutant channels reduced current threshold for firing an all-or-none action potential by approximately 50%, hyperpolarized the voltage threshold by 5.6 mV, increased the spike height by 11%, and prolonged the action potential duration by 25%. However, the mutant did not affect resting membrane potential, input resistance or after-hyperpolarization potential. The effects of the mutation on gating properties of $Na_v1.8$ are being evaluated. These observations suggest that expression of this novel *SCN10A* variant enhances the response of small DRG neurons to external stimuli compared with wild-type $Na_v1.8$, which contributes to the pain phenotype in this individual, and enlarges the spectrum of mutations in $Na_v1.8$ that are linked to human pain disorders.

Disclosures: J. Huang: None. **P. Zhao:** None. **J.G.J. Hoeijmakers:** None. **M.M. Gerrits:** None. **G.P. Lauria:** None. **C.G. Faber:** None. **I.S.J. Merkies:** None. **S.D. Dib-Hajj:** None. **S.G. Waxman:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.14/B25

Topic: B.04. Ion Channels

Support: R01- NS053792

T32-NS07491

F31-NS077700-01

Epilepsy Foundation Predoctoral Fellowship

Title: Hlf is a genetic modifier of epilepsy caused by sodium channel mutation

Authors: *N. HAWKINS, J. KEARNEY;
Pharmacol., Northwestern Univ., Chicago, IL

Abstract: In the United States, nearly 3 million people have epilepsy. Several types of human epilepsies, including Dravet syndrome, can be attributed to mutations identified in voltage-gated sodium channel genes. Genetic epilepsy pedigrees illustrate how the same sodium channel mutation can result in heterogeneous phenotypes. This suggests that other factors, possibly genetic, modify the primary mutation and alter disease severity. The genetic basis of epilepsy can be studied using mouse models. Frequently, the genetic strain background can change the disease phenotype, supporting a contribution of genetic modifiers in epilepsy. The Scn2aQ54 (Q54) transgenic mouse model has a strain-dependent epilepsy phenotype. Q54 mice on the C57BL/6J strain exhibit delayed seizure onset and improved survival compared to [B6xSJL/J]F1-Q54 mice. We mapped two dominant modifier loci the influence Q54 seizure susceptibility. Hlf (hepatic leukemia factor) was identified as a strong candidate modifier gene at one locus by RNA-Seq analysis. Hlf was hypothesized to modify the Q54 seizure phenotype by contributing to strain-specific variation in PDXK and PLP levels, enzymes involved in the pyridoxine pathway and neurotransmitter metabolism. An Hlf targeted knockout mouse model assessed the modifier potential. Hlf KO/+ was crossed with Q54 to generate Hlf KO/+;Q54 mice, which were then crossed with Hlf KO/+ to generate Hlf KO/KO;Q54 test mice and controls. Spontaneous seizure frequency was measured during 30 minute observations at 3 and 6 weeks of age and survival was monitored until 9 weeks. We observed a significant difference in seizure frequency ($p<0.0236$) and survival ($p<0.002$) between Hlf KO/KO;Q54 mice and controls. To determine if direct

modulation of the pyridoxine pathway could alter their phenotype, Q54 mice were fed a pyridoxine deficient diet for 6 weeks. At 3 weeks of age, mice were randomly assigned modified or control diet for 6 weeks. At 9 weeks, spontaneous seizure frequency was determined, as described above. Q54 mice maintained on a pyridoxine deficient diet exhibited elevated seizure frequency ($p<0.0120$) and decreased survival ($p<0.002$) compared to controls. To determine if Hlf could modify other epilepsies, Hlf KO/+ mice were crossed with the Scn1aKO/+ Dravet syndrome mouse model to examine the effect on the early lethality phenotype. Hlf KO/+;Scn1aKO/+ offspring exhibited a significant lifespan reduction ($p<0.0001$) compared to Scn1aKO/+ controls. Together these results demonstrate that Hlf is a genetic modifier of epilepsy caused by voltage-gated sodium channel mutations and that modulation of the pyridoxine pathway can also influence phenotype severity.

Disclosures: **N. Hawkins:** None. **J. Kearney:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.15/B26

Topic: B.04. Ion Channels

Title: Nav1.7 selective sodium channel inhibitor CNV1014802 attenuates stimulus-evoked action potential activity in hyperexcitable DRG neurons expressing inherited erythromelalgia mutations

Authors: ***S. D. DIB-HAJJ**[1], M. ESTACION[1], D. DERJEAN[2], V. MORISSET[2], S. TATE[2], S. G. WAXMAN[1];
[1]Dept Neurol, Neurosci & Regen Res. Ctr, Yale Sch. Med., West Haven, CT; [2]Convergence Pharmaceuticals an affiliate of Biogen Idec, Cambridge, United Kingdom

Abstract: Genetic and functional studies have shown that sodium channel Nav1.7, which is highly expressed in dorsal root ganglion (DRG) neurons, is an important player in pain signaling in humans; gain-of-function mutations of Nav1.7 are known to underlie pain syndromes such as inherited erythromelalgia (IEM) and paroxsysmal extreme pain disorder (PEPD), and loss-of-function mutations cause channelopathy-associated congenital indifference to pain (CIP). This suggests that selective pharmacological blockade of Nav1.7 could result in significant reduction of pain. CNV1014802 is a novel small molecule state-dependent blocker that preferentially inhibits Nav1.7 sodium channels. Here we report the effects of CNV1014802 on rat DRG

neurons expressing the hNav1.7-F1449V mutant channel which has been identified in IEM patients. CNV1014802 had a clear effect on action potential current threshold in DRG neurons expressing F1449V mutant channels, which increased from a mean of 134 pA (pre-drug) to 203 pA during exposure to 1 uM drug. We also evaluated the effect of CNV1014802 on the response to 1000 msec stimuli in the subpopulation capable of firing at a high rate (>5Hz). Treatment of transfected DRG neurons with CNV1014802 reduced the firing frequency of these neurons. The increase of threshold and the reduction of peak firing rate in response to CNV1014802 suggest favorable clinical responses to this compound in patients with IEM.

Disclosures: **S.D. Dib-Hajj:** None. **M. Estacion:** None. **D. Derjean:** A. Employment/Salary (full or part-time):; Convergence Pharmaceuticals an affiliate of Biogen Idec. **V. Morisset:** A. Employment/Salary (full or part-time):; Convergence Pharmaceuticals an affiliate of Biogen Idec. **S. Tate:** A. Employment/Salary (full or part-time):; Convergence Pharmaceuticals an affiliate of Biogen Idec. **S.G. Waxman:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

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Program#/Poster#: 35.16/B27

Topic: B.04. Ion Channels

Support: NIH/NIEHS-T32ES007254

NIH Grant R01MH095995

NIH Grant R01ES015991

Title: Isoform- and age-dependent effects of deltamethrin on voltage-gated sodium channel expression and function

Authors: ***T. F. JAMES**[1], J. P. MAGBY[2], F. LAEZZA[1], J. R. RICHARDSON[2];
[1]Univ. of Texas Med. Br., Galveston, TX; [2]Rutgers Univ., Piscataway, NJ

Abstract: Pyrethroids have become the most common class of insecticides in the past decades, and their primary mode of action is to prevent inactivation of opened voltage gated sodium channels (Na_v), which results in increased excitability that can lead to permanent illness or even death. However, the details of acute and chronic exposure to pyrethroids remain poorly understood—effects beyond the delay of inactivation remain to be explored. Here we present

data demonstrating that acute exposure to the pyrethroid insecticide deltamethrin in developing Long-Evans rats induces down-regulation of voltage-gated sodium channel expression that is associated with increased calpain cleavage or 20S proteasomal processing based on age. Further, repeated exposure of postnatal rats to deltamethrin resulted in decreased sodium channel levels and hippocampal glutamate release in an age- and isoform-dependent manner. Furthermore, electrophysiological data demonstrate an isoform-specific effect of acute deltamethrin exposure on Na_v function in human embryonic kidney cells (HEK293) with variances between the magnitudes of induced persistent current and directionality of peak current densities mediated by the neuronal $Na_v1.1$ and $Na_v1.6$ channels. Some properties, such as the voltage-dependence of activation, remain identical across the two isoforms, indicating that deltamethrin may have multiple binding sites and/or is dependent on differences in Na_v primary sequences in order to exert its effect. Collectively, these data indicate that deltamethrin has differential effects depending on Na_v channel type, which may have implications for certain isoforms known to be involved in neuropsychiatric disorders. Additionally, these data have implications for reported age-related susceptibility to pyrethroid neurotoxicity. Funding source: NIH/NIEHS-T32ES007254 (TFJ), NIH Grant R01MH095995 (FL), R01ES015991 (JRR)

Disclosures: T.F. James: None. **J.P. Magby:** None. **F. Laezza:** None. **J.R. Richardson:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.17/B28

Topic: B.04. Ion Channels

Title: Nav1.7 inhibitor reduces stimulus-evoked action potential activity from small diameter trigeminal ganglion neurons

Authors: *M. R. ESTACION[1,2], D. DERJEAN[3], S. TATE[3], V. MORISSET[3], S. WAXMAN[1,2]; [1]Neurol, Yale Univ. Sch. of Med., New Haven, CT; [2]Ctr. for Neurosci. and Regeneration Res., Veteran Affairs Med. Ctr., West Haven, CT; [3]Convergence Pharmaceuticals, Cambridge, United Kingdom

Abstract: Genetic and functional studies have shown that Nav1.7 is an important player in pain signaling in humans. Nav1.7 is highly expressed in sensory neurons of dorsal root ganglion and trigeminal ganglion (TG) neurons and sets the gain on firing, and is considered an attractive target for development of next-generation analgesics. Here we report the effects of a novel state-

dependent Nav1.7 selective blocker, CNV1014802, in rat TG neurons. We evaluated the effect of CNV1014802 on TG neuron excitability by assessing the effect of graded concentrations of the compound on threshold and evoked spiking activity of small diameter TG neurons. CNV1014802 increased current threshold in all neurons. There was a clear dose-response effect on threshold, with 1 uM of CNV1014802 increasing threshold by 90 pA, 10uM increasing threshold by 130 pA and 100 uM increasing threshold markedly so that most cells did not fire even at 1000 pA stimulus current. While most small TG neurons fire one or two action potentials in response to a 1000 msec stimulus, a subpopulation of cells fire at a higher rate (>5Hz). CNV1014802 reduced the firing frequency of these neurons. The inhibitory effect of CNV1014802 on excitability of TG neurones provides a potential mechanism supporting the recent efficacy of the compound demonstrated in a trigeminal neuralgia phase II clinical study.

Disclosures: M.R. Estacion: None. **D. Derjean:** A. Employment/Salary (full or part-time):; Convergence Pharmaceuticals. **S. Tate:** A. Employment/Salary (full or part-time):; Convergence Pharmaceuticals. **V. Morisset:** A. Employment/Salary (full or part-time):; Convergence Pharmaceuticals. **S. Waxman:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.18/B29

Topic: B.04. Ion Channels

Title: DV21 decreases excitability of cortical pyramidal neurons and acts in epilepsy

Authors: *P. SUN[1], X. LI[2];
[1]Zhejiang Univ., Zhejiang, China; [2]Zhejiang Univ., Hangzhou Zhejiang, China

Abstract: Epilepsy is a disabling neurological disorder and affects about 1% of the population of all ages and often requires lifelong medication. However, nearly 30% affected individuals are refractory to current pharmacological treatment, so, it is necessary to develope new antiepileptic drugs. As we all know, natural products are important source of new drugs (about 60% drugs come from natural molecules directly or indirectly). We extracted a small natural molecule from plants in our country, which is named DV21 and detected to have remarkable antiepileptic activity. First, in the individual level, we find DV21 can decrease the onset time, latency of death time, and also decrease the mortality in the mouse model of epilepsy. Then, to prove it in the cell level, we find the number of action potential are reduced in the pyramid neuron after applying

the DV21 in acute cortical slices. Now we are investigating the mechanisms underlying the antiepileptic activity of DV21.

Disclosures: P. Sun: None. **X. Li:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.19/B30

Topic: B.04. Ion Channels

Support: NIH Grant R01 NS053792

NIH Grant R01 NS032387

Title: Strain-dependent differences in voltage-gated sodium currents in the Scn2aQ54 mouse model of epilepsy

Authors: C. H. THOMPSON, *J. A. KEARNEY, A. L. GEORGE, Jr.;
Pharmacol., Northwestern Univ., Chicago, IL

Abstract: A spectrum of genetic epilepsy is associated with mutations in genes encoding voltage-gated sodium channels. Mouse models have been valuable for elucidating mechanisms of epileptogenesis associated with sodium channel mutations. In the *Scn2a*^{Q54} model, a severe seizure phenotype is caused by increased persistent sodium current conferred by transgenic expression of an engineered mutation in $Na_V1.2$. Importantly, epilepsy severity in *Scn2a*^{Q54} mice exhibits a strong strain-dependence. When bred congenically onto the C57BL/6J background, *Scn2a*^{Q54} (B6.Q54) mice have a mild phenotype compared to animals that have been intercrossed for a single generation with SJL/J mice (F1/Q54), which are more severely affected. We used a combination of whole-cell voltage and current clamp recording on acutely dissociated hippocampal pyramidal neurons to investigate the basis for this strain dependence. Whole-cell voltage clamp recordings of voltage-gated sodium channels revealed that neurons from F1.Q54 animals exhibited a larger persistent current (expressed as a % of peak sodium current) compared to B6.Q54 animals (1.1 ± 0.2% vs 0.4 ± 0.1%, n = 6-10), while there was no differences in whole-cell current density. Additionally, neurons from F1.Q54 animals showed a more depolarized voltage dependence of inactivation compared to neurons from B6.Q54 animals, suggesting that these channels are more resistant to inactivation. Neither voltage-dependence of

activation, nor recovery from inactivation showed any strain-dependent differences. No differences were observed between the biophysical properties of sodium currents measured from wild-type mice of the two background strains. Finally, whole-cell current clamp recording revealed that neurons from both B6.Q54 and F1.Q54 animals show spontaneous action potential firing, but F1.Q54 neurons exhibited a higher level of activity compared to B6.Q54 neurons (6.5 ± 1.5 Hz vs 2.5 ± 0.5 Hz, n = 8-9). Neurons from F1.Q54 animals also showed greater evoked activity compared to B6.Q54 neurons. These data suggest that the strain-dependent phenotype observed for the *Scn2a*^{Q54} model is driven, at least in part, by divergent levels of sodium channel dysfunction between strains that confer different levels of neuronal hyperexcitability.

Disclosures: C.H. Thompson: None. **J.A. Kearney:** None. **A.L. George:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.20/B31

Topic: B.04. Ion Channels

Title: Biophysical and pharmacological properties of human Nav1.9 stably expressed in HEK 293 cells

Authors: *Z. LIN, S. SANTOS, K. M. PADILLA, D. PRINTZENHOFF, N. A. CASTLE; Biol., Pfizer Inc., Durham, NC

Abstract: Recent human genetic studies have identified gain of function mutations of the sensory neuron expressed voltage dependent sodium channel Nav1.9, which are associated with either complete insensitivity or hypersensitivity to pain. Thus, Nav1.9 is as an attractive target for development of new pharmacological treatments for pain. Despite its pathophysiological importance, little is known about Nav1.9, partly due to the historical difficulty of achieving functional expression of the recombinant channel. Here we report the generation, biophysical and pharmacological characterization of a HEK-293 cell line stably expressing the recombinant human Nav1.9 alpha subunit along with beta1 and beta2 accessory subunits. Examination of cell line using whole cell patch clamp revealed a slowly inactivating tetrodotoxin insensitive inward current (29 ± 9 pA/pF) with midpoint voltages of activation (-56.6± 0.5 mV) and inactivation (-46.7 ± 0.9 mV) following 100 ms voltage steps. Nav1.9 current density increased in the presence of GTP-gamma-S in the pipette solution, which is inconsistent with previous reports that Nav1.9 can be regulated by G protein coupled receptor modulation. Nav1.9 currents were inhibited by

established sodium channel blockers, but potency was considerably less than reported for other sodium channel subtypes. For example, IC_{50} (midpoint inhibition ± 95% CI in μM) for amitriptyline = 30 ± 5; lidocaine = 377 ± 56; mexiletine = 271 ± 36 and tetracaine = 38 ± 5. In other sodium channel subtypes, these inhibitors have been shown to interact with the local anesthetic binding (LA) site in the pore. We mutated the equivalent LA site residues of Nav1.9 (F1592A/Y1599A) and found that potencies for the inhibitors were reduced by 3 to 15 fold. This suggests that the inhibitors interact with the local anesthetic binding site, despite their low potencies observed for inhibition of wild type Nav1.9. The results from this study show that stable expression of Nav1.9 in HEK cells is possible, and the resulting cell line recapitulates the properties of native Nav1.9 currents in sensory neurons.

Disclosures: Z. Lin: None. **S. Santos:** None. **K.M. Padilla:** None. **D. Printzenhoff:** None. **N.A. Castle:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.21/B32

Topic: B.04. Ion Channels

Title: Interplay between CRMP2 phosphorylation and SUMOylation determines NaV1.7 trafficking

Authors: *A. MOUTAL, E. T. DUSTRUDE, X. YANG, Y. WANG, M. KHANNA, R. KHANNA;
pharmacology, Univ. of Arizona, Tucson, AZ

Abstract: Trafficking of voltage-gated sodium channel NaV1.7 remains poorly understood. Post-translational modifications of NaVs and/or auxiliary subunits and protein-protein interactions have been posited as NaV-trafficking mechanisms. We recently reported that modification of the axonal collapsin response mediator protein 2 (CRMP2) by a small ubiquitin-like modifier (SUMO) controls both NaV1.7 trafficking and currents (Dustrude et al., J. Biol. Chem. 288: 24316-31 (2013)). Because CRMP2 functions are regulated by its phosphorylation state, we investigated the possible interplay between phosphorylation and SUMOylation of CRMP2 on NaV1.7. Phosphorylation of CRMP2 by cyclin dependent kinase 5 (Cdk5) was necessary for maintaining NaV1.7 surface expression and current density. Loss of either CRMP2 SUMOylation or phosphorylation decreased binding to NaV1.7. Preventing both CRMP2

modification events simultaneously was not synergistic, suggesting that NaV1.7 co-opts both pathways to be functional. However, CRMP2 phosphorylation was obligatory for its SUMOylation. Loss of either CRMP2 SUMOylation or phosphorylation triggered NaV1.7 internalization in a clathrin-dependent manner involving the HECT domain-containing E3 ubiquitin ligase Nedd4-2 as well as other endocytosis adaptor proteins. Our findings identify a novel mechanism for selective regulation of NaV1.7.

Disclosures: **A. Moutal:** None. **E.T. Dustrude:** None. **X. yang:** None. **Y. Wang:** None. **M. Khanna:** None. **R. Khanna:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.22/B33

Topic: B.04. Ion Channels

Support: NIH grant, 2RO1HL071165

Title: Disease mutations affecting calmodulin and FGF13 interaction with Nav1.2 voltage-dependent sodium channel increase persistent sodium current

Authors: *H. YAN, C. WANG, G. S. PITT;
Dept. of Medicine, Ion Channel Res. Unit, Duke Univ. Med. Ctr., Durham, NC

Abstract: Whole exome sequencing studies have identified SCN2A, which encodes the neuronal voltage-gated Na+ channel NaV1.2, as one of the most commonly mutated genes associated with autism and intellectual disabilities. Several of the mutations cluster within the intracellular C terminal domain (CTD), the binding site for calmodulin (CaM) and members of the fibroblast growth factor homologous factor (FHF) family. Both CaM and FHFs are potent modulators of various aspects Na+ channel currents. Whether disease mutations within the NaV1.2 CTD affect Na+ currents because of disruption of interaction with (and consequent modulation by) CaM or FHFs is not known. Here, we tested the consequences on voltage-gated Na+ currents and on in the interaction with CaM or FHFs of several disease mutations (H1853R and R1918H associated with epilepsy; and R1902C associated with familial autism) within the NaV1.2 CTD. Binding studies with recombinant proteins showed that all three mutations reduced the affinity of the CTD for CaM and/or FGF13 (one of four FHF family members). Using a heterologous expression system, we showed that all three mutations affect various Na+ current

parameters_including steady-state inactivation the amount of the persistent “late” Na+ current. The reduced affinity for CaM and/or FGF13 appeared to be causative for the effects on Na+ currents since over-expression of the CaM and/or FGF13 “rescued” the disease-associated defect. Thus, our data suggest that CaM and FHFs are essential components of neuronal voltage-gated Na+ channels and that disease mutations may lead to altered Na+ currents through reduced interaction with the critical channel modulators.

Disclosures: H. Yan: None. **C. Wang:** None. **G.S. Pitt:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.23/B34

Topic: B.04. Ion Channels

Support: Innovative Development Award, UC Davis

Title: Modulation of the cardiac sodium channel Nav1.5 by the antiepileptic agent lacosamide

Authors: N. ELIA, X. XIONG, S. DHILLON, P. CHEN, A. MONTALVO, S. TANG, D. H. FELDMAN, *C. LOSSIN;
Neurol., UC Davis - Sch. of Med., Sacramento, CA

Abstract: Sodium channel blockers are the first line of defense in controlling seizures, but inhibition of voltage-gated sodium (Nav) channel activity may have undesired effects throughout the nervous system, heart, and muscle, due to the similarity of structure and function in all Nav variants of excitable tissues. Lacosamide (LCM) is approved therapy for adult partial seizures and its likely mechanism of action is the enhancement of Nav channel slow inactivation. The literature contains electrophysiological characterizations of LCM’s effect on neuronal Nav such as Nav1.2, Nav1.3, Nav1.7, etc., but the impact on cardiac Nav1.5 is mentioned only anecdotally. To get a better understanding of LCM action in the heart, we tested LCM (Ontario Chemicals) on whole-cell voltage-clamped HEK cells stably transfected with human Nav1.5. Our data show that, in presence of the drug, Nav1.5 entered slow inactivation much more readily, requiring less and shorter depolarization, and it recovered at a significantly slower pace than when LCM was absent. At high concentrations, the shift in the voltage dependence of slow inactivation was dramatic, effectively disabling approximately half of all Nav1.5 channels, even without depolarization. Contractility assays on cardiomyocytes showed complete but reversible

cessation of rhythmic beating between 30 and 100 μM LCM. Since pharmacokinetic studies suggest a patient serum concentration nearing 30 μM, our results indicate that LCM-induced enhancement of Nav1.5 slow inactivation is a plausible contributor to the observed cardiac effects, and suggests that there may be a narrow window of dosage safety, which if exceeded, may precipitate cardiac events.

Disclosures: **N. Elia:** None. **X. Xiong:** None. **S. Dhillon:** None. **P. Chen:** None. **A. Montalvo:** None. **S. Tang:** None. **D.H. Feldman:** None. **C. Lossin:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.24/B35

Topic: B.04. Ion Channels

Support: Regione Autonoma della Sardegna, Prog CRP3_63LR 7/2007-Bando 2010

Title: Modulation of hyperpolarization-activated cation currents (Ih) by ethanol in rat hippocampal CA3 pyramidal neurons and influence on neuronal excitability

Authors: V. LICHERI[1], G. TALANI[2], A. A. GORULE[1], L. FIRINO[1], L. COCCO[1], G. BIGGIO[1], *E. SANNA[1];
[1]Univ. Cagliari, Monserrato 09042, Italy; [2]of Neurosci., Natl. Res. Council, Monserrato, Italy

Abstract: Hyperpolarization-activated and cyclic nucleotide-gated channels (HCN) are expressed in different neuronal populations where they play a crucial role in fundamental neurophysiologial functions including neuronal pacemaker activity, dendritic integration, and membrane excitability. Recent studies have reported that ethanol (EtOH) is able to modulate Ih mediated by HCN in VTA and hippocampal interneurons. Because robust Ih are also present in CA3 pyramidal neurons, we here investigated whether the action of EtOH on CA3 excitability could be correlated with its possible interaction with HCN. To evaluate whether Ih were altered by EtOH, we initially tested a low (20 mM) and higher (60 and 80 mM) concentrations of EtOH which were bath applied for 10-15 min under voltage-clamp conditions in acute hippocampal slices from Sprague Dawley rats. Bath perfusion of 20 mM EtOH induced a significant increase in Ih amplitude with respect to control, whereas, 60 and 80 mM produced an opposite effect, with a significant reduction of Ih amplitude. Because the activity of HCN is considered a contributing factor in the fine-tuning of neuronal excitability, we also studied the effect of EtOH

on action potential firing in hippocampal CA3 pyramidal neurons. Firing in CA3 pyramidal cells is regulated by an intrinsic cellular mechanism, and recently, it was shown that the majority of pyramidal cells in the hippocampal CA3 field support persistent firing only in the presence of the muscarinic receptor agonist carbachol. Carbachol-induced firing in CA3 pyramidal neurons was increased following perfusion of 20 mM EtOH, while 80 mM reduced the frequency of action potentials. In addition, bath perfusion of ZD-7288 (20 μM), selective HCN blocker, as well as the non-selective blocker CsCl (5 mM), completely suppressed action potential firing. Overall these findings suggest that the biphasic modulatory action of EtOH on HCN-mediated Ih may contribute to the effects of EtOH on the excitability of CA3 pyramidal cells. Funded by grant from Regione Autonoma della Sardegna, Prog CRP3_63LR 7/2007-Bando 2010.

Disclosures: **V. Licheri:** None. **G. Talani:** None. **A.A. Gorule:** None. **L. Firino:** None. **L. Cocco:** None. **G. Biggio:** None. **E. Sanna:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.25/B36

Topic: B.04. Ion Channels

Support: NIHGM Grant 2SC1GM084854-05A1

NCRR Grants 5R25GM061838-15, 2G12-RR003051

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NIGMS Grant 5T34GM007821

Title: Changes in Ih current channel's subunits 2 and 4 during the expression of cocaine sensitization

Authors: *C. E. MARÍA-RÍOS[1,2], A. MONTIEL-RAMOS[1,2], B. SANTOS-VERA[1], A. VAQUER-ALICEA[1], R. VÁZQUEZ-TORRES[1], C. A. JIMÉNEZ-RIVERA[1];
[1]Physiol., Univ. Of Puerto Rico Med. Sci. Campus, San Juan, Puerto Rico; [2]Biol. Dept., Univ. of Puerto Rico Río Piedras Campus, San Juan, Puerto Rico

Abstract: Cocaine abuse is known to induce neurobiological adaptations in the Mesocorticolimbic (MCL) system; a brain reward circuit composed of interconnected regions

that regulate pleasure and motivation. Some of these adaptations include changes in the excitability of the neurons in the MCL system, which can contribute to the development and expression of cocaine sensitization. Cocaine sensitization is the progressive escalation of psychomotor responses that results with repeated cocaine administration. The Hyperpolarization-Activated Cyclic-Nucleotide Current (Ih), is a pronounced mixed cation current present in MCL neurons. The potential regulatory role of Ih current in neuronal excitability is subjected to the expression of Hyperpolarization-Activated Cyclic-Nucleotide gated channel (HCN) subunits. Ih may play a significant role in the behavioral responses to cocaine. Previously, our laboratory demonstrated that protein expression of the HCN_2 subunit, was increased in the Ventral Tegmental Area (VTA), Nucleus Accumbens (NAcc), Hippocampus (HIP), and Prefrontal Cortex (PFC) after the development of cocaine sensitization. However, protein expression of the HCN_4 subunit was significantly decreased only in the VTA. The purpose of the present study was to elucidate if protein expression levels of HCN_4 and HCN_2 subunit observed during the development of cocaine sensitization persist after a 7-day cocaine-withdrawal period, or if normal expression was reinstated. Sprague Dawley male rats (250g) received intraperitoneal cocaine (15mg/kg) or 0.9% saline injections for 7 days. Locomotor activity was recorded for one hour. Following a 7-day cocaine withdrawal period, rats were sacrificed and tissue micro-punches from VTA, NAcc, HIP and PFC collected and put through protein extraction and western blot analysis. Our results show a significant 61% decrease in protein expression of HCN_4 subunit in the HIP (sample T-test; $p< 0.05$). In addition, preliminary data shows a 71% decrease in protein expression of HCN_4 in PFC (n=4). These results may suggest possible changes emerging during the cocaine-withdrawal period that could be essential for the maintenance of the neuroadaptations that are present during cocaine addiction. Protein expression of the HCN_2 subunit during the expression of sensitization is still being explored.

Disclosures: C.E. María-Ríos: None. **A. Montiel-Ramos:** None. **B. Santos-Vera:** None. **A. Vaquer-Alicea:** None. **R. Vázquez-Torres:** None. **C.A. Jiménez-Rivera:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.26/B37

Topic: B.04. Ion Channels

Support: NIH Grant MH065339

NSF Grant DMS-1120952

Title: Determining the distribution and functional role of HCN channels in a collision sensitive neuron

Authors: *E. SUNG[1], R. B. DEWELL[2], S. J. COX[1], F. GABBIANI[2];
[1]Computat. and Applied Mathematics, Rice Univ., Houston, TX; [2]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: The Lobula Giant Movement Detector (LGMD) neuron in locusts is an identified visual neuron that responds vigorously to approaching objects on a collision course with the animal. The LGMD serves as a strong model to study single neuron computations. It has been studied extensively for the past 50 years, and its role in locust escape behavior has been well characterized. The LGMD consists of three dendritic subfields denoted as subfields A, B, and C. Subfield A receives ~15,000 retinotopic excitatory inputs whereas subfields B and C receive inhibitory inputs. Current clamp recordings with pharmacological manipulations have revealed the presence of the inward-rectifying current Ih mediated by HCN channels. However, the distribution and functional role of these HCN channels in the LGMD has yet to be fully characterized. To study such questions, we use computational methods to model the membrane dynamics and to ascertain the role of the HCN channels. We perform 3D morphological reconstructions of the LGMD to better characterize the size and geometry of the LGMD. We repeat this procedure over several LGMD neurons from different animals. Then, we incorporate these reconstructions into a Hodgkin-Huxley type multi-compartmental model. Using this model, we first investigate how the HCN channels are distributed in the LGMD's dendritic subfields. We do this by fitting our model to dual potential experimental recordings in order to recover an estimated guess of the HCN channel distribution. To study the functional role of the HCN channels in the LGMD, we simulate incoming retinotopic synaptic inputs in the presence and absence of HCN channels, and observe the change in response of the LGMD. We show that the HCN channels play a role in shaping LGMD responses to incoming synaptic inputs.

Disclosures: E. Sung: None. **R.B. Dewell:** None. **S.J. Cox:** None. **F. Gabbiani:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.27/B38

Topic: B.04. Ion Channels

Support: NIH Grant MH065339

NSF Grant DMS-1120952

Title: Influence of active dendrites on membrane resonance and temporal synchrony in a looming sensitive neuron

Authors: *R. B. DEWELL[1], F. GABBIANI[1,2];
[1]Dept of Neurosci., Baylor Col. of Med., Houston, TX; [2]Rice Univ., Houston, TX

Abstract: How a neuron integrates complex spatial and temporal patterns of synaptic inputs is critical for the computations it performs. Synaptic integration depends in turn on the active conductances localized within a neuron's dendritic arbor. We use a well-studied looming sensitive neuron in the locust that integrates ~15,000 retinotopically organized excitatory synaptic inputs as a model system. This neuron, the lobula giant movement detector (LGMD) responds to approaching objects or their two dimensional analogues, called looming stimuli, in a manner that is largely invariant to the object's shape, texture, contrast and approach angle. Experimental evidence suggests that both I_h and I_M contribute to complex, nonlinear processing within the LGMD, which allows reliable detection of approaching objects. These currents can produce resonance within single neurons that results in increased responses to inputs within a particular frequency range. In addition to this ability to band-pass filter synaptic inputs, I_h has also been shown to increase the temporal synchrony of inputs, by reducing the phase lag of signals as they propagate from distal dendrites toward the site of spike initiation. As the spatio-temporal pattern of synaptic inputs into the LGMD is important both for the computations carried out within the neuron and the animal's escape behavior, this neuron represents an attractive model to investigate the influence of active conductances on the resonance and temporal filtering of synaptic inputs. To study these questions, we used chirp currents in single and dual recording experiments *in vivo* combined with pharmacological manipulations of M and h currents. This system allows us to not only investigate the role of active conductances for dendritic integration, but also to ground these data in the context of an ecologically important neural computation.

Disclosures: R.B. Dewell: None. **F. Gabbiani:** None.

Poster

035. Sodium Channels, Hearing and Communication Neuroscience, and Other Non-Selective Cation Channels

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 35.28/B39

Topic: B.04. Ion Channels

Support: NIH Grant F32 MH103964-01

NIH Grant R01 MH095948-03

Title: Alterations in cerebellar stellate cell hyperpolarization-activated currents following fear learning

Authors: *K. L. CARZOLI, J. LIU;
Cell Biol. and Anat., LSUHSC, New Orleans, LA

Abstract: Along with its canonical role in motor coordination, the cerebellum has been shown to be involved in associative learning. One form of associative learning, fear conditioning, has been found to alter synaptic transmission and connectivity in a cerebellar lobule-specific manner. Most studies of fear conditioning have focused on how learning alters synaptic transmission, but few reports have actually investigated the influence of learning on membrane excitability. A neuron's intrinsic properties are determined by ion channels in the membrane, the alteration of which can affect neuronal activity and output. Unlike most voltage-gated channels, hyperpolarization activated cyclic nucleotide-regulated (HCN) channels are nonselective cation channels that activate with hyperpolarization and deactivate with depolarization. Furthermore, these channels can produce a small inward current near a neuron's resting membrane potential, and thereby influence intrinsic membrane properties. After fear leaning, it has been shown that there is an increase in inhibitory transmission in the cerebellum. Cerebellar stellate cells are inhibitory interneurons that express HCN channels, an alteration of which could influence their membrane excitability and the activity that they output onto their principal Purkinje cells. Here, we investigated the effects of fear learning on hyperpolarization-activated currents (Ih) using voltage clamp recordings. We found that fear conditioning reduced the amplitude of Ih in cerebellar stellate cells. Moreover, we found that this reduction in Ih resulted in an increase in neuronal input resistance. These learning-induced changes were specific to vermal lobules V/VI, and changes in Ih were reversed following extinction. We concluded that changes in Ih were due to associative fear learning because when mice underwent a control procedure in which conditioned and unconditioned stimuli were explicitly unpaired, these leaning-induced changes did not occur. Together, our findings aid in our understanding of how learning can alter membrane excitability in the cerebellum.

Disclosures: K.L. Carzoli: None. **J. Liu:** None.

Poster

036. Presynaptic Structure and Neurotransmitter Release I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 36.01/B40

Topic: B.06. Neurotransmitter Release

Support: INSERM

NIH Grant K99NS087110

NS35915

Title: The supramammillary-dentate gyrus pathway: evidence for a unique glutamate and gaba co-transmission

Authors: L. CASTILLO[1,2], A. IVANOV[1,2], A. GHESTEM[1,2], E. KROOK-MAGNUSON[3], I. SOLTESZ[4], C. BERNARD[1,2], *M. ESCLAPEZ[1,2];
[1]INSERM UMR 1106 - INS, Marseille Cedex05, France; [2]Aix-Marseille Univ., Marseille, France; [3]Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN; [4]Anat. and Neurobio., Univ. of California Irvine, Irvine, CA

Abstract: The supramammillary nucleus (SuM) provides substantial projections to the hippocampal formation. This hypothalamic structure is involved in the regulation of hippocampal theta rhythm and therefore plays a crucial role in the control of several hippocampal-dependent cognitive functions as well as emotional behavior. In rat previous studies demonstrate that neurons from the lateral region of the SuM (SuML) which innervates the supragranular layer of the dorsal dentate gyrus (DG) and, to a much lesser extent the ventral DG co-express markers for both glutamatergic (vesicular glutamate transporter 2; VGluT2) and GABAergic (glutamate decarboxylase 65, GAD65 and the vesicular GABA transporter, VGAT) neurotransmission and establish asymmetric and symmetric synapses on dentate granule cells. In this study, we examine the physiological features of this potential co-transmission using an *in vitro* optogenetic experimental approach performed in VGLUT2-Cre mutant mouse (Jackson's Laboratory) expressing Chanelrhodopsin (ChR2, excitatory opsin) within SuML neurons and their axon terminals after stereotaxic injection of an AAV5 cre-dependent ChR2 viral vector (UNC Vector Core, Desseroth's laboratory) into the SuML. We confirm in this mutant mouse, the dual neurotransmitter phenotype of SuML neurons, described in rat. We then demonstrate in hippocampal slices, that light activation of SuML axon terminals expressing channelrhodopsin2 induced simultaneously a fast glutamate postsynaptic current (PSC) and a slower GABA PSC onto a single granule cell. The GABA-A receptor antagonist gabazine (10 μM) blocked light-evoked GABA PSC and increased (or reveal) the glutamate PSC. The AMPA-receptor antagonist NBQX (10 μM) selectively blocked the light-evoked glutamate current without modifying the GABA PSC. Our findings indicate that Glutamate and GABA are co-released monosynaptically from SuML axon terminals. The functional role of such unique glutamate and

GABA co-transmission by SuML neurons that could simultaneously depolarize and hyperpolarize dentate granule cells remains to be demonstrated.

Disclosures: **L. Castillo:** None. **A. Ivanov:** None. **A. Ghestem:** None. **E. Krook-Magnuson:** None. **I. Soltesz:** None. **C. Bernard:** None. **M. Esclapez:** None.

Poster

036. Presynaptic Structure and Neurotransmitter Release I

Location: Hall A

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Title: Presynaptic NMDA receptors act via RIM1αβ to control the readily-releasable pool in layer-5 pyramidal neurons

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Abstract: Presynaptic NMDARs (preNMDARs) of unclear function have been found at several central synapse types. Although it is known that they increase vesicle release probability, precisely how is unknown. We recorded evoked release onto layer-5 pyramidal cells (PCs) using paired recordings in acute slices of juvenile mouse visual cortex. PreNMDARs could affect evoked release in several different ways: by directly altering release probability, by increasing the readily releasable vesicle pool (RRP) size, or by upregulating RRP replenishment rate. We used Schneggenburger-Neher's approach to examine the RRP, depleting it with 14 spikes at 30 Hz every 80 seconds. We first compared preNMDAR blockade to external [Ca2+] reduction.

The GluN2B-specific NMDAR blocker Ro25-6981 (Ro) 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, lowered [Ca2+] decreased RRP size (-27% ± 4%, n = 9, $p < 0.05$ vs. controls) but not replenishment rate (7.8% ± 7%, $p = 0.33$). Next, we extended the Tsodyks-Markram short-term plasticity model to include preNMDARs and tuned it to control and Ro conditions. The model indicated that both vesicle usage and recovery from depression were downregulated by Ro, and that preNMDARs improve signal-to-noise ratio during high-frequency firing.Finally, we explored how preNMDARs signal by hypothesizing a need for RIM1. In slices from heterozygote RIM1αβ conditional KO mice, we found decreased EPSC amplitudes (32 ± 4 pA, n = 36 vs. controls 54 ± 10 pA, n = 22, $p < 0.01$) and increased paired-pulse facilitation (-0.30 ± 0.05 vs. controls -0.47 ± 0.03, $p < 0.001$), consistent with a low baseline release probability. Ro blockade left EPSC amplitude in RIM1αβ KO indistinguishable from mock wash-in (96% ± 4%, n = 12 vs. 98% ± 10%, $p = 0.88$), but reduced it in RIM1αβ flox controls (52% ± 10%, n = 6, ANOVA $p < 0.01$). Similarly, Ro had no distinguishable effects on RRP size ($p = 0.68$) or replenishment rate ($p = 0.83$) in KO mice vs. mock wash-in (pooled: 1.6% ± 10% and -9.8% ± 4%), but reduced both in RIM1αβ flox controls (-69%±5%, $p < 0.001$ and -42% ± 9%, $p < 0.01$, ANOVAs). Cortical preNMDARs thus need RIM1αβ to regulate the RRP. We conclude that during high-frequency firing, preNMDARs signal via RIM1αβ to upregulate the RRP replenishment rate, thereby increasing the release probability indirectly. As a consequence, preNMDARs boost high-frequency neurotransmission and improve the signal-to-noise ratio.

Disclosures: **T. Abrahamsson:** None. **R.P. Costa:** None. **K. Buchanan:** None. **D. Elgar:** None. **A. Blackman:** None. **J. Oyrer:** None. **A. Tudor-Jones:** None. **M. van Rossum:** None. **P.J. Sjöström:** None.

Poster

036. Presynaptic Structure and Neurotransmitter Release I

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Program#/Poster#: 36.03/B42

Topic: B.06. Neurotransmitter Release

Title: Investigation of calcium currents upon overexpression of αCav2.1 in the Calyx of Held

Authors: ***M. LUEBBERT**[1], B. DAS[1,3], W. DONG[1], K. GOFF[1], R. RICHARD[1], N. KAMASAWA[2], S. M. YOUNG, Jr.[1];

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Abstract: Previous studies on cultured hippocampal neurons proposed the existence of voltage-gated Ca2+ channel (VGCC) subtype preferring slots at the active zone (AZ). It was proposed that these slots are binding sites with different capacities for each VGCC subtype that interact with the α-subunit of VGCCs. Two different kinds of slots are expressed at the AZ: 1) Cav2.2 (N-type) channel slots exclusively binding Cav2.2 VGCCs with an unlimited capacity and 2): Cav2.1 (P/Q-type) preferring slots with limited capacity that still accept Cav2.2 VGCCs (Cao et al., 2004; Cao et al., 2010). The calyx of Held is a giant presynaptic terminal, forming a glutamatergic axosomatic synapse with the principal cells of the medial nucleus of the trapezoid body (MNTB) in the auditory brainstem. During development (P7-P11) the composition of VGCCs at the calyx consists of R, N, and P/Q-type. However, by hearing onset (mice ~P11) the calyx exclusively expresses P/Q at the synaptic terminal. Recently, it was shown that the number of P/Q-type channels in calyx of Held AZs varies strongly (2-73; Nakamura et al., 2015), implying that AZs themselves have variable slots for P/Q-type VGCCs. However, it remains unclear if AZs have variable numbers of slots or if AZs express similar numbers of slots that remain unsaturated. To answer this question, we used a helper-dependent adenoviral vector (HdAd) to overexpress αCav2.1 in the calyx. Expression was driven by the high level expression cassette pUNISHER. Subsequently, we conducted whole-cell patch clamp recordings to investigate the effect of αCaV2.1 overexpression on presynaptic Ca2+ currents. The results obtained from these recordings will be discussed.

Disclosures: **M. Luebbert:** None. **B. Das:** None. **W. Dong:** None. **K. Goff:** None. **R. Richard:** None. **N. Kamasawa:** None. **S.M. Young:** None.

Poster

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Support: NEUROSCAFFOLDS-FP7-NMP-604263

CARBONANOBRIDGE-ERC-2008-227135

PRIN-MIUR n. 2012MYESZW

Title: Artificial nanostructures mimicking the extracellular environment: sculpting neurotransmission during maturation of hippocampal synapses

Authors: *N. P. PAMPALONI[1], D. SCAINI[2], S. BOSI[2], M. PRATO[2], L. BALLERINI[3]; [1]SISSA, Trieste, Italy; [2]Univ. of Trieste, Trieste, Italy; [3]sissa, Trieste, Italy

Abstract: Neurons sense physical and chemical features of the extracellular environment that are then translated into appropriate actions. This ability has been exploited in modern neuroscience to manufacture nanostructured growth scaffolds with biomimetic cues useful for enabling key biological tasks in cultured tissues (Dvir et al, 2010; Shao and Fu, 2014; Battiston et al, 2014). Multi-walled carbon nanotubes (MWCNT) are nanostructures constituted by sheets of graphene rolled up to form hollow tubes (Iijima 1991). Over the last decade MWCNTs were shown to be an ideal interface to grow excitable cells such as neurons and cardiomiocytes (Mattson et al, 2000; Dvir et al, 2010; Fabbro et al, 2013). The extracellular environment when artificially reconstructed by MWCNTs induces synaptogenesis in cultured neurons (Lovat et al, 2005; Mazzatenta et al, 2007; Cellot et al, 2009, 2011). In practice, the physical and chemical properties of MWCNTs provide potent biomimetic signals to neurons and significantly favor the construction of monosynaptic connections among pairs of neurons when synaptic networks mature *in vitro* (Cellot et al, 2011). There are currently no reports on the effects that immobilized MWCNTs exert on the cell membrane equilibria, however several studies targeted the interaction between lipidic membrane and MWCNTs in suspension (Monticelli et al, 2009). Here we investigate the impact of MWCNTs meshwork on the synaptic homeostasis of cultured neurons at different stages of growth. By patch-clamp and immunofluorescence microscopy we investigated whether the ability of such nanomaterials to modulate excitatory synaptic transmission was related to alteration in cell membrane lipids, in particular we focused on cholesterol, known to balance presynaptic vesicle release (Wasser et al, 2007; Ramirez and Kavalali, 2011). We exclude the presence of major MWCNT-induced cholesterol changes in the membrane of cultured neurons. However, we unmasked MWCNT ability to shape synapse formation and the synaptic mode of transmission of glutamatergic connections during *in vitro* development. Finally, we described for the first time network activity homeostasis upon long term MWCNT interfacing. Battiston, Biomaterials (2014) 35:4465. Cellot, J.Neurosci. (2011) 31:12945. Cellot Nat Nanotech (2009) 4:126. Dvir T Nat Nanotech (2011) 6:13. Fabbro ADDR (2013) 65:2034. Iijima Nature (1991) 354:56. Lovat, NanoLetters (2005) 5:1107. Mattson J Mol Neurosci (2000)14:175. Mazzatenta, J Neurosci (2007) 27:6931. Monticelli Soft Matter (2009) 5:4433. Ramirez Curr Opin Neurobiol (2012) 21:275. Shao Adv Mater (2014) 26:1494. Wasser J Physiol (2007) 579:413.

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Poster

036. Presynaptic Structure and Neurotransmitter Release I

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Title: Disturbances in presynaptic active zone structure-function relationships in Lambert-Eaton myasthenic syndrome

Authors: *T. B. TARR[1], J. MA[2], M. DITTRICH[2], S. D. MERINEY[1];
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Abstract: Although the mammalian neuromuscular junction (NMJ) is normally a very reliable synapse, there are disease states in which this reliability can be drastically reduced. One such example is Lambert-Eaton myasthenic syndrome (LEMS), an autoimmune disease that reduces neurotransmitter release at the NMJ and leads to muscle weakness in patients. The reduction in neurotransmitter release is thought to occur because of an autoantibody-mediated removal of a fraction of the presynaptic voltage-gated Ca2+ channels (VGCCs) that trigger release of neurotransmitter-containing vesicles. Despite the well accepted theory that a reduction in the number of presynaptic VGCCs causes the physiological properties observed in LEMS NMJs, large LEMS-induced changes in short-term plasticity at mouse-model synapses led us to examine this hypothesis in greater detail. To explore this issue, we used a combination of electrophysiological recording, calcium imaging, and MCell modeling techniques. We find that changes in short-term plasticity cannot be explained by a simple reduction in the number of VGCCs. First, we pharmacologically blocked neurotransmitter release to a similar extent as observed in our LEMS model (~75% reduction in quantal content) using ω-agatoxin IVA, but this pharmacological block did not generate short-term plasticity characteristics that matched those of LEMS NMJs. Second, we conducted computer modeling studies based on our previously developed 3-dimensional reaction-diffusion MCell computer model of the mouse NMJ that accurately predicts experimentally observed quantities, such as quantal content, release latency and short-term plasticity characteristics. Using this MCell model we tested the hypothesis that removal of VGCCs can predict the short-term plasticity observed at LEMS model NMJs. As with our physiological recordings, we find that simple VGCC removal is not sufficient to explain the experimentally observed changes in facilitation under LEMS conditions. Therefore, using this model we are currently testing the functional role of additional changes to

active zone organization in the observed LEMS NMJ physiology. These changes include alterations in the distance between VGCCs and synaptic vesicles and the LEMS-induced up-regulation of additional VGCC types that might not be positioned within the highly organized active zone structure. Lastly, we have used our newly developed VGCC gating modifier (GV-58) as an experimental tool to test these short-term plasticity mechanisms both physiologically and in our MCell model.

Disclosures: **T.B. Tarr:** None. **J. Ma:** None. **M. Dittrich:** None. **S.D. Meriney:** None.

Poster

036. Presynaptic Structure and Neurotransmitter Release I

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Program#/Poster#: 36.06/B45

Topic: B.06. Neurotransmitter Release

Title: Reduced endogenous calcium buffering speeds active zone calcium signaling

Authors: *S. J. HALLERMANN[1], I. DELVENDAHL[1], J. LUKASZ[1], C. BAADE[1], V. MATVEEV[2], E. NEHER[3];
[1]Univ. Leipzig, Leipzig, Germany; [2]New Jersey Inst. of Technol., Newark, NJ; [3]Max-Planck-Institute for Biophysical Chem., Goettingen, Germany

Abstract: Fast synchronous neurotransmitter release at the presynaptic active zone is triggered by local calcium signals, which are confined in their spatio-temporal extent by endogenous calcium buffers. However, it remains elusive how rapid and reliable calcium signaling can be sustained during repetitive release. Here, we established quantitative two-photon calcium imaging in cerebellar mossy fiber boutons, which fire at exceptionally high rates. We show that endogenous fixed buffers have a surprisingly low calcium-binding ratio (~15) and low affinity, whereas mobile buffers have high affinity. Experimentally constrained modeling revealed that the reduced endogenous buffering accelerates the clearance of calcium at the active zone during repetitive firing. Measuring calcium signals at different distances from active zones with ultra-highresolution confirmed our model predictions. Our results lead to a novel concept that reduced calcium buffering speeds active zone calcium signaling, suggesting that the strength of endogenous calcium buffering limits the rate of synchronous synaptic transmission.

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Poster

036. Presynaptic Structure and Neurotransmitter Release I

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Title: Novel functions of ADF/cofilin-dependent actin dynamics in neurotransmitter release and mouse behavior

Authors: *M. B. RUST[1,2], A. GÖRLICH[2], M. WOLF[2], A.-M. ZIMMERMANN[2], C. GURNIAK[3], M. SASSOÈ-POGNETTO[4], E. FRIAUF[2], W. WITKE[3];
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Abstract: Actin depolymerizing proteins of the ADF/cofilin family are essential for actin dynamics, which is critical for synaptic function. Two ADF/cofilin family members, namely ADF and cofilin1, are highly abundant in the brain and present at excitatory synapses. By exploiting gene-targeted mice we have shown previously that cofilin1 is critical for dendritic spine morphology, synaptic plasticity, associative learning, and anxiety (Rust et al., EMBO J, 2010; Goodson et al., PLoS Genet, 2012). Conversely, inactivation of ADF in mice did not interfere with synapse morphology or physiology and mutant mice performed normal in paradigms of associative learning (Görlich et al., PLoS One, 2011). Interestingly, we found elevated cofilin1 levels in synaptic structures from ADF mutant mice suggesting i) that cofilin1 can compensate for the loss of ADF and ii) that ADF and cofilin1 have overlapping synaptic functions. To test these suggestions, we genetically removed ADF together with cofilin1 from synapses. In double mutant mice, synaptic actin dynamics was impaired and more severely affected than in single mutants (Wolf et al., Cereb Cortex, 2015; Rust et al., Exp Cell Res, 2015). The resulting cytoskeletal defects affected the organization and mobilization of synaptic vesicles

and thereby impaired neurotransmitter release. Enhanced glutamate release in the striatum caused attention deficit/hyperactivity disorder (ADHD)-like behavioral abnormalities in double mutants that were not present in single mutants, including hyper-locomotion and a calming effect of psychostimulants (Zimmermann et al., Biol Psychiatry, 2015). Together, our data revealed novel and redundant functions for ADF and cofilin1 in neurotransmitter release and mouse behavior. Moreover, they highlight the relevance of actin dynamics for presynaptic mechanism and associated defective actin dynamics with the etiology of ADHD.

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Poster

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Title: Distinct molecular signatures of NE and ATP containing vesicles in vascular sympathetic nerves

Authors: ***J. WALIA**[1], B. LI[1], C. GERSHOME[1,2], D. POBURKO[1,2];
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Abstract: Sympathetic vericosities release neurotransmitters including ATP and NE into the neuroeffector junction to regulate vascular smooth muscle cell contraction and ultimately blood pressure. Despite advances in our knowledge of presynaptic signalling and organization in cultured sympathetic neurons, few studies have characterized presynaptic molecular details in the sympathetic terminals in intact blood vessels. Our work focuses on whether ATP and norepinephrine (NE) are co-stored in the same vesicles or in distinct pools of vesicles and/or varicosities. Using immunohistochemistry (IHC) and epifluorescence microscopy, we previously found that the vesicular monoamine transporter 2 (VMAT2) and vesicular nucleotide transporter (VNUT), which load NE and ATP in to vesicles respectively, are segregated within varicosities. Here we report IHC analyses to characterize the association of VNUT with other vesicular proteins: Synaptic Vesicle 2A (SV2A), Synaptophysin (Syp) and Synaptotagmin-1 (Syt1), the Ca2+ sensor for NE containing vesicles. Reports studying vas deferens suggested that SV2A

localizes to all vesicles of sympathetic nerve terminals, whereas Syp might preferentially associate with VNUT-containing vesicles. Clusters (fluorescent puncta) of labelled vesicles were analyzed for fluorescent intensity and their distance to neighbouring puncta of other proteins using ImageJ. Morphologically, VNUT labelled small and highly punctate pools of vesicles that were smaller than typical varicosities (typically <500 nm), whereas Syt1 labelled pools of vesicles roughly the same size as vericosities. Syp- and SV2A-labelled vesicle pools were of intermediate size between VNUT and Syt1, with labelling consisting of small, round puncta within a varicosity. Visually, VNUT was anti-colocalized with all but a small subpopulation of vesicles labelled by Syt1, SV2A and Syp. In contrast, Syp and SV2A extensively colocalized with Syt1, but they also labelled small pools of vesicles lacking Syt1. The separation between the perimeters of VNUT and Syp, SV2A or Syt1 puncta averaged 0.1 μm, whereas Syt1 and SV2A or Syp consistently overlap by at least 0.1 μm. Correlations in puncta intensities for Syt1 and SV2A (r=0.23) or Syp (r=0.36) and VNUT-Syp (r=0.29) were high relative to VNUT and SV2A (r=0.02) or Syt1 (r=-0.05). Thus we conclude that Syp and SV2A are preferentially localized with Syt1 to NE containing vesicles, but also associate with a small subpopulation of ATP containing vesicles. Moreover, ATP containing vesicles appear to lack Syt1, which may contribute to differential sympathetic release of ATP and NE as a function of stimulation frequency and duration.

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Poster

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Title: All-or-none axonal Ca^{2+} dynamics in recurrent circuits of the hippocampus

Authors: ***I. RAN**[1], R. W. TSIEN[2];
[1]NYU Neurosci. Inst., NYU Langone Med. Ctr., New York, NY; [2]NYU Neurosci. Inst., NEW YORK, NY

Abstract: An action potential (AP) is assumed to travel actively and reliably along axons and trigger Ca2+ entry into nerve terminals without fail, leaving all-or-none regulation of release probability (Pr) in the hands of downstream SNARE proteins. Using viral delivery in mature

hippocampal slice cultures and two-photon imaging of fast genetically-encoded Ca2+ sensor, GCaMP6f, we explored the dynamics of axonal Ca2+ transients (CaTs) evoked by single AP stimulation of CA3 pyramidal neurons in organotypic slices studied at 30°C. CaT amplitudes followed a right-skewed distribution (>700 boutons). Strikingly, 1AP CaTs imaged along single axons failed intermittently on repeated (30) trials at neighbor boutons. Conservative histogram analysis of CaT amplitude revealed a bimodal distribution with clear separation of CaT successes and failures. CaT failures rarely spread across an entire branch, suggesting that they were not due to conduction failure. Notably, prolonging AP duration with the K+ channel blocker 4-aminopyridine (100 μM) reduced CaT failure rate and increased both their amplitude and duration. Finally, a subset of axons exhibited functionally silent boutons that were unresponsive to single trial AP stimulation but responded to a stronger 50 AP train pattern. In these silent boutons, a visible CaT signal evolved within 5-10 APs, possibly due to recruitment of Ca2+ channels or build-up of residual Ca2+. Altogether, our finding uncover large all-or-none variations in axonal CaT dynamics and leave ample room for Pr regulation, upstream of SNAREs, via Ca2+ channel modulation.

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Poster

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Title: Impaired endosomal system mediated by Cathepsin D produces defects in GABAergic synaptic transmission

Authors: *X. LI[1,2], Y. LI[1], H. YU[1], Z. ZHANG[1], Y. LIU[1], L. QIN[1], Z. XU[1], Z. GAO[1], S. DUAN[1];
[1]Zhejiang Univ., Zhejiang, China; [2]Nantong Univ., Nantong, China

Abstract: The role of endosomal/lysosomal system for proper brain function is underscored by many neurological disorders. Cathepsin D (CathD, ctsd, EC 3.4.23.5) is a soluble aspartic endopeptidase in this system, that mediates the cleave of structural and functional proteins and

peptides. However, how Cathepsin D affects the function of neurons and synapses remains unknown. Here we show that Cathepsin D deficiency mice recapitulated hyperactivity with the absence of anxiety by the open field (OF) and elevated plus maze (EPM) tests. Increased pentylentrazol (PTZ) induced epilesy seizure susceptibility was measured in knock out mice. Loss of ctsd also demonstrated the degenerative loss of neurons and proliferation of reactive astrocytes with increased levels of GFAP in the cotex and hippocampus using immunocytochemistry. Cultured hippocampal neurons lacking ctsd showed reduced GABAergic synapses and inhibitory/excitatory synaptic ratio while Glutamergic synaptic marker did not affected.Cathepsin D deficiency is accompanied by significant changes in hippocampal synaptic plasticity, due to reduced GABAergic synaptic strength and impaired GABAergic synapses RRP. FM4-64 dye staining and destaining in micro-island cultured GAD-GFP neuron revealed specifically inhibition effects on GABAergic synaptic vesicle exocytosis pathway. These data suggested that Cathepsin D may be critical for normal function of GABAergic neurons and subtle imbalance between GABAergic and Glutamergic synaptic circuit may contribute to numerous neurological phenotypes.

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Poster

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Title: Hydrogen peroxide modulates synaptic transmission in ventral horn neurons of the rat spinal cord

Authors: M. OHASHI, *T. KOHNO, N. OHASHI, T. HIRANO, K. WATANABE, H. SHOJI, N. ENDO;
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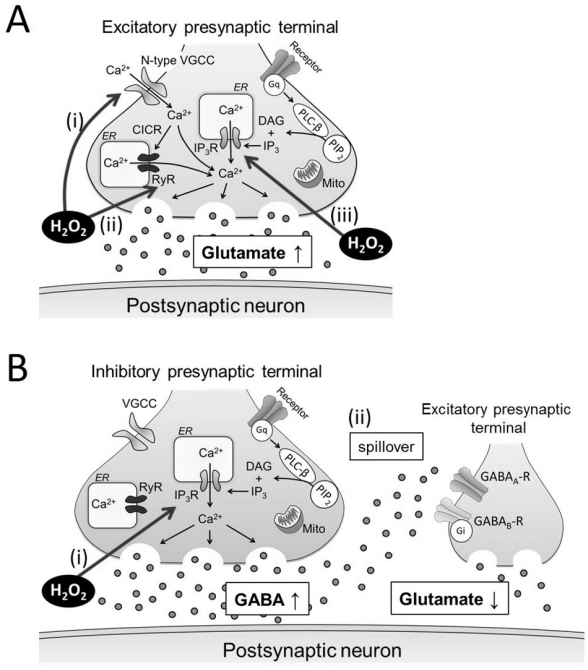
Abstract: Excessive production of reactive oxygen species (ROS) is a critical component of the cellular and molecular pathophysiology of many central nervous system (CNS) disorders, including trauma, ischemia-reperfusion injury, and neurodegenerative diseases. Hydrogen peroxide (H2O2), an abundant ROS, modulates synaptic transmission and contributes to neuronal damage in the CNS; however, the physiological role of H2O2 in spinal cord ventral horn (VH) neurons remains poorly understood, despite reports that these neurons are highly vulnerable to oxidative stress and ischemia. This was investigated in the present study using a whole-cell path-clamp approach in rats. We found that elevated H2O2 levels increased the release of glutamate and γ-aminobutyric acid (GABA) from excitatory and inhibitory presynaptic terminals, respectively, of VH neurons. The former was induced in part by an increase in Ca2+ influx through N-type voltage-gated calcium channels (VGCCs) as well as by ryanodine receptor (RyR)- and inositol triphosphate receptor-mediated Ca2+ release from the endoplasmic reticulum (ER); in inhibitory presynaptic neurons, increased IP3R-mediated Ca2+ release from the ER increased GABAergic transmission, which served to rescue VH neurons from excessive release of glutamate from presynaptic terminals. These findings indicate that inhibiting N-type VGCCs or RyRs may attenuate excitotoxicity resulting from increased glutamatergic activity while preserving the neuroprotective effects of GABA, and may therefore represent a novel and targeted strategy for preventing and treating H2O2-induced motor neuron disorders.



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Poster

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Title: Synaptic functions of γRIM proteins

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Abstract: The large members of the RIM protein family, RIM1α/β and RIM2 α/β, are centrally involved in the cytomatrix of the presynaptic active zone. Through multiple interactions with other active zone proteins as well as synaptic vesicle proteins they are involved in several steps of presynaptic neurotransmitter release. αRIMs are important for maintaining sufficient and localized calcium entry to trigger fast release of vesicles and for maintaining synaptic vesicles in a release-ready state. The RIM protein family contains two additional small isoforms, RIM3γ and RIM4γ, which are composed of only the RIM-specific C-terminal C2B domain and an isoform specific N-terminus. The C2B domain of all RIM proteins has been suggested to bind to the β subunit of the presynaptic N- and P/Q-type voltage gated Ca2+-channels and to modulate their opening times. However, the synaptic function of γRIMs in neurons has not been studied in detail up to now. We addressed this question by analyzing glutamatergic synaptic transmission in native brain slices of newly generated RIM3γ and RIM4γknockout (KO) mice. Field potential recordings of Schaffer collateral-evoked synaptic activity of CA1 pyramidal neurons indicate reduced paired-pulse facilitation (PPF) in RIM4γ KO mice which is in contrast to the previously reported increase in PPF in RIM1α KO mice. The function of the presynaptic release machinery is assessed by an approach that allows us to quantitatively estimate calcium binding properties of the release machinery and the relative release probability. We use ω-conotoxin GVIA, an N-type calcium channel blocker, to reduce presynaptic calcium entry to a certain and constant fraction and to quantify the resulting relative decrease in synaptic transmission while varying the extracellular calcium concentration. The differential sensitivity of field potentials to the ω-conotoxin GVIA suggests a differential contribution of N- and P/Q-type calcium channels at the

active zone of RIM1α and RIM4γ KO mice. Furthermore, RIM4γ KO mice develop a strong phenotype of episodic ataxia and seizure-like episodes at around P21, accompanied by reduction in weight and body size. In contrast, RIM3γ KO mice do not exhibit an overt phenotype. In summary, the data point at a prominent role of RIM4γ for maintaining normal neuronal network function.

Disclosures: S. Ferrando-Colomer: None. **K. Michel:** None. **D. Dietrich:** None. **S. Schoch:** None.

Poster

036. Presynaptic Structure and Neurotransmitter Release I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 36.13/B52

Topic: B.06. Neurotransmitter Release

Support: NIH Grant NS078165

Title: α-Synuclein interacts with Hsc70: a possible mechanism underlying the synaptic vesicle recycling defects in Parkinson's disease models

Authors: *S. M. BANKS[1], D. J. BUSCH[2], P. A. OLIPHINT[3], R. B. WALSH[4], J. M. GEORGE[5], E. M. LAFER[6], J. R. MORGAN[1];
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Abstract: Overexpression and abnormal aggregation of α-synuclein is linked to neurodegeneration in Parkinson's disease (PD). A growing body of evidence suggests that α-synuclein aggregation is prevalent at synapses, disrupting synaptic function in PD and other synucleinopathies. However, the underlying mechanisms causing the synaptic defects remain unclear. To address this, we utilize lamprey giant reticulospinal synapses, which are ideal for studying the acute effects of excess human α-synuclein on synaptic structure and function. Using this model, we previously showed that excess human α-synuclein causes an inhibition of synaptic vesicle recycling that affects both clathrin-mediated and bulk endocytosis. Here, we test the hypothesis that excess α-synuclein causes these recycling defects by binding to other core components of the endocytic pathway, thus inhibiting their function. Using GST-pull downs, we

tested for α-synuclein interactions with well-characterized proteins involved in clathrin-mediated endocytosis: AP2, clathrin, dynamin, synaptojanin, auxilin and Hsc70. The only interaction observed was a direct and specific interaction between α-synuclein and Hsc70, the clathrin uncoating ATPase at synapses. Hsp70 did not bind under the same conditions. PD-linked α-synuclein mutants A30P and A53T also bound to Hsc70. Mapping of the interaction revealed that the N-terminal domain of α-synuclein (a.a. 1-102) was sufficient for the interaction and the C-terminal 10kD of Hsc70 was required. The interaction is evolutionarily conserved, because lamprey γ-synuclein also bound to Hsc70. The N-terminal domain of α-synuclein folds into an alpha helix and readily binds to small vesicles containing acidic phospholipids such as phosphatidic acid (PA). Interestingly, α-synuclein binding to PA-containing vesicles was reduced in the presence of Hsc70, and Hsc70 recruitment to PA-containing vesicles was significantly increased in the presence of α-synuclein. These data suggest that the Hsc70-synuclein interaction also occurs on membranes and that the two proteins may affect each other's localization and function. As a preliminary test of this model, we evaluated whether excess α-synuclein affects clathrin uncoating at synapses, a process that is attributed to Hsc70 function. After treatment with excess human α-synuclein, synapses exhibited nearly a 4-fold increase in the number of clathrin-coated vesicles, suggesting that α-synuclein may bind Hsc70 and disrupt its function during vesicle recycling. Taken together, these findings provide one possible mechanism for how excess α-synuclein leads to synaptic defects in PD.

Disclosures: S.M. Banks: None. **D.J. Busch:** None. **P.A. Oliphint:** None. **R.B. Walsh:** None. **J.M. George:** None. **E.M. Lafer:** None. **J.R. Morgan:** None.

Poster

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Program#/Poster#: 36.14/B53

Topic: B.06. Neurotransmitter Release

Support: CIHR

Title: Distinct functional roles for P/Q- and N-type voltage-gated calcium channels in synchronous glutamate release

Authors: *S. CHAMBERLAND, A. EVSTRATOVA, K. TÓTH;
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Abstract: In presynaptic terminals, calcium elevations are shaped by several key parameters, including the properties, density, combination and the spatial location of VGCCs. These features allow presynaptic terminals to translate complex firing frequencies to postsynaptic signals by regulating the amount of neurotransmitter released. For example, the number of vesicles fusing to the membrane can be amplified through mechanisms such as synchronization of multivesicular release or recruitment of additional release sites. While synchronous release relies on both P/Q- and N-type VGCCs at hippocampal MF-CA3 synapses, the contribution of individual types of VGCCs to the mechanisms controlling neurotransmitter release remains unknown. To dissect the roles of P/Q and N-type VGCCs, we used random-access two-photon calcium imaging and electrophysiology in combination with electron microscopy. Our results show that calcium influx through P/Q- and N-type VGCCs differently influence glutamate release during a single action potential firing and repetitive activity through specialized calcium dynamics. First, two-photon calcium imaging in giant mossy fiber terminals revealed that P/Q-type VGCCs mediated a larger fraction of calcium elevations than N-type VGCCs for a single action potential. Consistent with calcium imaging data, P/Q-type VGCCs showed a significantly larger contribution to glutamate release than N-type VGCCs. However, this difference was dependent on the external calcium concentration, as decreasing the aCSF calcium concentration to 1.2 mM revealed a similar effect for both toxins. To investigate how calcium entry through N-type VGCCs can mediate a larger fraction of EPSCs in conditions of low release probability without changes in presynaptic calcium entry, we used a coefficient of variation (CV) analysis of single and train of stimuli. While blocking N-type VGCCs decreased the quantal size of EPSCs, blocking P/Q-type VGCCs reduced EPSC amplitude by reducing the number of active release sites. Furthermore, CV analysis revealed that application of ω-Agatoxin IVA or EGTA-AM had similar effects on short-term facilitation by eliminating the recruitment of additional release sites. Altogether, our results demonstrate the highly specialized roles of P/Q- and N-type VGCCs in neurotransmitter release. While N-type VGCCs are tightly coupled to calcium sensors and provide local calcium elevations, P/Q-type VGCCs are strategically involved to support global calcium elevations and recruit additional release sites during trains of activity.

Disclosures: **S. Chamberland:** None. **A. Evstratova:** None. **K. Tóth:** None.

Poster

036. Presynaptic Structure and Neurotransmitter Release I

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Topic: B.06. Neurotransmitter Release

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MRC Grant MR/K022105/1

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Title: Ultrastructural and functional fate of recycled vesicles in hippocampal synapses

Authors: ***S. REY**[1], C. SMITH[1], M. W. FOWLER[1], F. CRAWFORD[1], J. J. BURDEN[2], K. STARAS[1];
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Abstract: There is substantial interest in understanding the mechanisms of vesicle recycling that support sustained transmission at size-limited central synapses. The readily releasable pool (RRP) defines the population of synaptic vesicles that undergo privileged fusion, but the long-term fate of the vesicles recovered after endocytosis and their contribution to future RRP composition is not clearly established. Here, we exploit time-stamped electron microscopy approaches in native hippocampal synapses, as well as novel fluorescence methods, to track the positional and functional destiny of vesicles retrieved after RRP stimulation. We show that most vesicles are endocytosed near the active zone but are subsequently inserted randomly in the cluster volume with time, losing their preferential re-use status. These vesicles non-selectively queue, advancing towards the release site with further stimulation in a process that depends on actin turnover. However, a subset of the retrieved vesicle population operates differently, selectively re-clustering near the active zone and undergoing privileged re-release as part of the future RRP. We use a variety of protocols to examine the rules that underpin this differential behaviour. Heterogeneity in the fate of vesicles retrieved after RRP stimulation provides new understanding of the mechanisms that govern vesicle recycling at small central synapses and the origins of future pool composition.

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Poster

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Program#/Poster#: 36.16/B55

Topic: B.06. Neurotransmitter Release

Support: OCAST

COMAA Research Fund

Title: Role of reduced synaptobrevin 2 levels in age-related cognitive decline

Authors: *A. OROCK[1,2], S. LOGAN[2,3], W. E. SONNTAG[1,2,3], F. DEAK[1,2,3];
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Abstract: Introduction: One of significant socio-economic impacts from the rise of the elderly population worldwide is age related cognitive decline. Synaptic dysfunction is emerging as the major cause of cognitive impairment and dementia as synaptic plasticity is a central mechanism in learning and memory. Our laboratory and others have identified synaptobrevin-2, a SNAP Receptor (SNARE) protein as a key player in synaptic transmission and plasticity. Synaptobrevin-2 (syb2) is the major SNARE protein of synaptic vesicles (SV) which is highly expressed in the cerebral cortex and hippocampus, essential brain centers for spatial learning. Syb2 protein levels have also been shown to decrease with age but functional consequences of this lower expression are still elusive. We hypothesize that reduction of syb2 protein levels with age mediates age related cognitive decline. **Methods:**We have been using heterozygous syb2 knock-out mice which express syb2 at about the same level at 6 month-of-age as old (24 mo) wild-type animals. This is 50-60% of the protein levels found in young wild-type littermates ($p<0.01$, Student t-test). As a proof of concept, we are also using aged (>24 months) TgV2 animals which overexpress syb2 in this study. This will enable us know if overexpressing syb2 will rescue the phenotype seen in old animals with reduced syb2. We performed behavioral tests for spatial learning and memory using the radial-arm water maze (RAWM) and a complex cohabitate environment called Intellicage. Neuronal plasticity was assessed by long-term potentiation (LTP) assays on hippocampal slices. We used live fluorescence microscopy to measure the SV release rate in neuronal cultures. **Results:** The behavioral test with syb2 heterozygous resulted in maintained basic spatial memory acquisition for simple place finding (RAWM) but impaired learning in complex tasks in Intellicage. This impaired spatial memory was reflected in reduced CA1 hippocampal LTP and Syb2 heterozygous neurons have reduced SV release rates (~35% in 15 seconds, $p<0.01$ compared to wild-type controls). Importantly, aged animals overexpressing syb2 performed better in the RAWM task than wild-type suggesting that syb2 plays a major role in age related cognitive decline. **Conclusions:** To our best knowledge, this is the first study to demonstrate that syb2 levels have a causative role in synaptic failure and dementia, and our results suggest syb2 reduction with age mediates age-related cognitive decline.

Disclosures: A. Orock: None. **S. Logan:** None. **W.E. Sonntag:** None. **F. Deak:** None.

Poster

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Topic: B.06. Neurotransmitter Release

Support: NSERC RGPIN 326821

Title: Examining the role of Neurexins in the Formation and Maturation of Synapses between Hippocampal Neurons

Authors: D. P. QUINN[1], A. KOLAR[1], J. P. FAWCETT[2], *S. R. KRUEGER[3];
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Abstract: Neurexins are a diverse family of cell adhesion molecules that are expressed in CNS neurons and localize to presynaptic specializations. Neurexins mediate synaptic cell contact through extracellular interactions with postsynaptic adhesion molecules of the Neuroligin and LRRTM families. Studies suggest that neurexins have important roles in synapse formation and neurotransmitter release. To further investigate the role of neurexins in synaptic physiology, we used two approaches to disrupt neurexin function: 1) Overexpression of a dominant negative neurexin construct which lacks the extracellular domain necessary for trans-synaptic adhesion and 2) shRNA knockdown of all neurexins. By using a genetically encoded optical sensor for synaptic vesicle exocytosis called synaptophysin-pHluorin, we were able to examine the effect of neurexin perturbation on synaptic release at single synapses in dissociated hippocampal culture. We found that neurexin perturbation resulted in smaller readily releasable pool sizes at presynaptic compartments as well as a decrease in the likelihood with which individual vesicles in the pool undergo fusion in response to an action potential. We also find that neurexin disruption caused a decrease in active zone protein content and synaptic density; an effect that could be due to a decrease in synapse formation or an increase in synapse elimination. To address these possibilities we used time-lapse imaging of tagged pre- and postsynaptic proteins to assess the effect of neurexin perturbation on synapse formation and synapse stability. We found that axons with perturbed neurexin function retain the ability to form synapses, though these synapses were transient in nature and are eliminated at a much higher rate. Taken together, these experiments suggest that neurexins may be dispensable for synapse formation, but needed for the stabilization and maturation of functional synaptic contacts.

Disclosures: D.P. Quinn: None. **A. Kolar:** None. **J.P. Fawcett:** None. **S.R. Krueger:** None.

Poster

036. Presynaptic Structure and Neurotransmitter Release I

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Program#/Poster#: 36.18/B57

Topic: B.06. Neurotransmitter Release

Support: NIH/NEI: EY021195

Title: The PXDLS-binding cleft of Ribeye is required for normal synaptic ribbon function

Authors: C. LV, E. PRESCOTT, S. VIVIANO, J. SANTOS-SACCHI, *D. P. ZENISEK;
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Abstract: Tonic non-spiking cells of the retina and inner ear release neurotransmitter from vesicles tethered to specialized protein structures known as synaptic ribbons. The most abundant protein in the synaptic ribbon is Ribeye, but its role in synaptic ribbon formation and function is poorly understood. Ribeye arises from an alternative start site to the transcriptional co-repressor gene CtBP2, with the C-terminal half of Ribeye nearly identical to CtBP2. CtBP2 interacts with many proteins via its PXDLS-binding cleft. We investigated the importance of the PXDLS-binding cleft in retinal ribbon synapse function using zebrafish as a model system. To do so, we generated transgenic zebrafish overexpresssing wild-type Ribeye(a)-YFP and Ribeye(a)-YFP with a mutation to the PXDLS-binding cleft. Zebrafish overexpressing Ribeye(a) with a mutation to the PXDLS-binding domain exhibited a reduction in the electroretinogram (ERG) b wave, which was not observed in fish overexpressing wild-type Ribeye(a). Zebrafish *in vivo* hair cell recordings show that overexpressed Ribeye(a)-YFP cells exhibit no significant differences in exocytosis as measured by membrane capacitance changes. By contrast, PXDLS-binding cleft mutants exhibit an approximately 50% reduction in exocytosis in response to 3s step depolarizations to -10 mV. Electron microscopy of neuromast hair cells revealed that animals overexpressing binding-cleft mutant Ribeye exhibit morphologically normal ribbons. Together, our results suggest that the PXDLS-binding cleft is necessary for proper ribbon function.

Disclosures: C. Lv: None. **E. Prescott:** None. **S. Viviano:** None. **J. Santos-Sacchi:** None. **D.P. Zenisek:** None.

Poster

036. Presynaptic Structure and Neurotransmitter Release I

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Topic: B.06. Neurotransmitter Release

Support: GM09687304

Title: Sparse SCN VIP projections to the PVN consistent with paracrine signaling

Authors: *J. M. WEBB[1], C. MAZUSKI[2], E. HERZOG[2], C. WEICHSELBAUM[2], D. CAI[3], D. ROOSSIEN[3];
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Abstract: The mammalian suprachiasmatic nucleus (SCN), the body's master circadian pacemaker, consists of approximately 20,000 neurons in the ventral hypothalamus. Neurons within the SCN have been categorized into more than 20 cell types based on their neuropeptide expression. Among these, vasoactive intestinal polypeptide (VIP) neurons have been identified as critical for coordinating circadian rhythms within the SCN and body. VIP receptor 2 (VPAC2R) is known to be widely expressed in the paraventricular nucleus of the hypothalamus (PVN), an upstream regulator of circadian glucocorticoid release. However, little is known about the anatomy or physiology of VIP projections outside of the SCN. We hypothesized, based on the broad distribution and low expression of VPAC2R in the PVN, that VIP neurons would have large, divergent projections in the PVN. We injected Cre-dependent Brainbow mTFP/mCherry and TagBFP/eYFP viruses into the right SCN of VIP-Cre knockin mice. After two weeks, the brains were harvested, sectioned at 100μm and processed for 3-color immunohistochemistry using chicken anti-GFP, rabbit anti-mCherry, and guinea pig anti-mKate2 antibodies. Sections were imaged on a confocal microscope with 0.5μm optical sections, and traced using a novel ImageJ plugin. Preliminary results from ~600 reconstructed neuronal processes indicated that nearly 100% of VIP axons could be discriminated in the PVN and subPVN. We found that, of all the VIP neurons entering the PVN, about 20% have terminals within the PVN. Of these VIP neurons that synapse in the PVN, the majority typically have a single terminal within the PVN with a maximum of 10 terminals within the PVN per SCN VIP neuron. These synapses are evenly distributed across the PVN. In addition, SCN VIP neurons appear to make similar numbers and distributions of terminals in the ipsilateral and contralateral PVN. Our findings support the conclusion that VIP neurons form sparse terminals in the PVN and likely communicate through a paracrine signal. Supported by NIGMS.

Disclosures: **J.M. Webb:** None. **C. Mazuski:** A. Employment/Salary (full or part-time):; Washington University in St. Louis. **E. Herzog:** A. Employment/Salary (full or part-time):; Washington University in St. Louis. **C. Weichselbaum:** A. Employment/Salary (full or part-time):; Washington University in St. Louis. **D. Cai:** A. Employment/Salary (full or part-time):; University of Michigan. **D. Roossien:** A. Employment/Salary (full or part-time):; University of Michigan.

Poster

036. Presynaptic Structure and Neurotransmitter Release I

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Topic: B.06. Neurotransmitter Release

Support: NINDS grant NS014506

NINDS grant NS007158

Title: The structural basis of priming of docked synaptic vesicles at the frog's neuromuscular junction

Authors: *J. JUNG, J. A. SZULE, U. J. MCMAHAN;
Biol., Texas A&M Univ., College Station, TX

Abstract: Docked synaptic vesicles undergo priming before fusing with the presynaptic membrane to mediate synaptic impulse transmission. We searched by electron tomography for structural correlates of priming at active zones on the presynaptic membrane of axon terminals at frog neuromuscular junctions. In resting terminals, the contact area between docked vesicles and the presynaptic membrane varied >10-fold. Evoked synaptic transmission selectively reduced the frequency of vesicles having a large contact area. Contact area correlated with the force-dependent eccentricity of vesicle shape and the shortness of the active zone material macromolecules linking vesicles to presynaptic membrane components that include Ca^{2+}-channels. The findings lead to the conclusions that priming is a variable continuum of events imposing on each vesicle variable fusion probability and that it is regulated by force-generated shortening of active zone material macromolecules in dynamic equilibrium. These conclusions are consistent with the findings of others indicating that the SNARE core complex and its auxiliary proteins regulate priming as well as docking. Our approach may provide a way of establishing the degree of priming of any docked vesicle at any synapse under normal, experimental and disease conditions.

Disclosures: J. Jung: None. **J.A. Szule:** None. **U.J. McMahan:** None.

Poster

037. Synaptic Organization in Hippocampus

Location: Hall A

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Topic: B.07. Synaptic Transmission

Support: JSPS KAKENHI 15K00325

MEXT–Supported Program for the Strategic Research Fundation at Private Universities, 2013–2017

Title: Interaction of inputs in hippocampal granule cells with inhibitory connections

Authors: N. NAKAJIMA[1], H. HAYAKAWA[2], T. KITAJIMA[3], *T. AIHARA[1];
[1]Tamagawa Univ., Tokyo, Japan; [2]Kyoto Inst. of Technol., Kyoto, Japan; [3]Universiti Teknologi Malaysia, Johor Bahru, Malaysia

Abstract: The hippocampus integrates non-spatial information (such as objects and odors) and spatial information (places). The dentate gyrus is the gate for input-information to the hippocampus. Non-spatial information and spatial information are independently transported from the entorhinal cortices to separated sites, the distal dendrite and the medial dendrite of granule cells (GCs) within the molecular layer in the dentate gyrus, respectively. To investigate the interaction of those two inputs, response characteristics of distal and medial dendrites of GCs were independently measured at 0.1-40Hz of input frequency in a rat hippocampal slice where inhibitory input was blocked by application of picrotoxin, GABAergic receptors antagonist. From those experimental data, a multi-compartment GC model with dynamic synapses was developed using NEURON simulator. Model simulations were performed using the model which reproduced the response characteristics of the dendrites of a GC. As the results, when the random and theta burst inputs were simultaneously applied to the respective dendrites, the pattern discrimination for theta burst input to medial dendrites that caused slight GC activation was enhanced in the presence of random input to distal dendrites. These results suggest that the temporal pattern discrimination of spatial information is originally involved in a synaptic characteristic in GCs and is enhanced by non-spatial information input to distal dendrites. Furthermore, to investigate a role of inhibitory neurons for the interaction between medial and distal inputs in the dentate gyrus, response characteristics were also physiologically measured in the presence of inhibitory inputs, without picrotoxin. In addition, model simulation was also performed. It was found that the local inhibitory network played to maintain magnitudes of EPSP for successive inputs to distal dendrite at high-frequency (25-40Hz). The result suggests that inhibitory inputs may support the enhancement of input information to medial dendrites by sustaining responses for input sequence to distal dendrites.

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Poster

037. Synaptic Organization in Hippocampus

Location: Hall A

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Topic: B.07. Synaptic Transmission

Support: ANPCyT PICT2010-1110

FOCEM

ANPCyT PICT2013--0182

Title: High frequency filters with variable gain in the dentate gyrus generated by differential inhibition onto developing and mature granule cells

Authors: M. B. PARDI[1], M. B. OGANDO[1], A. F. SCHINDER[2], *A. MARIN-BURGIN[1]; [1]Ibioba-conicet-Partner Inst. of the Max Planck, Buenos Aires, Argentina; [2]Fundacion Inst. Leloir, Buenos Aires, Argentina

Abstract: Adult neurogenesis generates pools of constantly renewing immature granule cells (GC) with unique processing properties. We have previously showed that immature GC have lower thresholds of activation than the rest of the GC from the circuit, already mature. In the present work we address the question of how immature and mature granule cells process complex stimuli in the form of trains of pulses at different frequencies, and how activation profiles are determined by the recruitment of inhibitory and excitatory circuits. To study activation of immature newly born cells in the adult hippocampus, we inject a retrovirus in the dorsal hippocampus of adult mice to label dividing cells. Four weeks later, we prepare acute hippocampal slices from the injected mice, where we can recognize the immature four week old adult born granule cells (4wpiGC). By simulating the afferent medial perforant path with a monopolar electrode and recording from 4wpi GC and mature GC with loose-patch and whole-cell configurations we studied spiking and evoked excitatory and inhibitory currents after 1 Hz, 10 Hz, 20 Hz and 40 Hz trains of stimulation. Results show that 4wpi GC are activated at higher levels than mature GC at every frequency of stimulation. Moreover, 4wpiGC are more efficient in reproducing the frequency of stimulation with their spiking. Activation levels strikingly diminish at higher frequencies, showing that both populations of GC act as low-pass filters. Main

differences in the activation profiles are dictated by the inhibitory circuits. Inhibition determines activation by affecting the excitation/inhibition balance the GC receives after the stimulation. Thus, activity arriving to the hippocampus at different frequencies activates two populations of neurons with variable frequency filters, immature cells, with wide range of responses, that are reliable transmitters of the incoming frequency, and mature neurons, with narrow responses to frequency and sparse activity.

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Poster

037. Synaptic Organization in Hippocampus

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Support: Margaret Olivia Knip Foundation

Title: Balance between excitatory and inhibitory synaptic inputs in principal neurons in the hippocampal CA1 and Dentate Gyrus regions resulting from electrical stimulation in an *ex vivo* mouse model

Authors: *S. D. DE KNECHT[1], P. CHAMEAU[2], W. WADMAN[2];
[1]Swammerdam Insitute of Life Sci., [2]Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: Afferent electrical stimulation of Schaffer collaterals (SC) evokes a combination of excitatory and inhibitory synaptic responses in pyramidal neurons (PYRs) of the CA1 region. Afferent stimulation of the perforant path (PP) evokes a similar response in granule cells (GCs) of the DG region. These principal neurons were recorded under voltage clamp and linear decomposition was used to separate the excitatory and inhibitory conductance. The excitatory response preceded the inhibitory response in PYRs (Δt: 3.8 ± 1.1 ms) as well as in GCs (Δt: 3.9 ± 0.8 ms). In GCs the peak amplitude of the inhibitory conductance was smaller (47%) than that of the excitatory conductance, while these values were not different in PYRs. In GCs the rise time of the excitatory conductance was faster (78%) than that of the inhibitory conductance. Inhibitory conductance in GCs also had a larger decay time (time constant 17 ± 2 ms) than can be explained by the kinetics of the IPSC, suggesting population recruitment. This observation also explains the short duration of the reconstructed post synaptic potential in GCs. The I-E balance was defined as the fractional inhibition calculated from each conductance integrated over

the first 50 ms. This balance was slightly larger in PYRs (53 ± 3 %) than in GCs (43 ± 3%). Increasing stimulus intensity shifted the balance in both regions towards inhibition, suggesting a stronger recruitment of interneurons. Repetitive stimulation (5 - 20 Hz) gradually reduced the excitatory conductance in GCs, but not in PYRs. The inhibitory conductance decreased with each stimulus in both neuron types. Thus repetitive stimulation results in a stronger reduction in balance in PYRs than in GCs, with potential consequences for the stability of the circuit. Our results indicate that activation of a single afferent pathway recruits a circuit response in the targeted neurons with distinct dynamics with at least two different time scales. Most neuronal networks seem to maintain a well-defined balance between excitation and inhibition. However, the detailed kinetics of the underlying conductances and intensity dependent circuit recruitment shape the fast synaptic response, while differences in use-dependent adaptation shape the time course of the I-E balance at a larger time scale. The two regions studied play a sequential role in hippocampal signal processing; the clear differences in dynamics has consequences for the overall characteristics of signal processing in this region.

Disclosures: S.D. De Knecht: None. **P. Chameau:** None. **W. Wadman:** None.

Poster

037. Synaptic Organization in Hippocampus

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Research Enhancement Program at the LSU School of Medicine

Title: Frequency-dependent input processing in hippocampal CA1 pyramidal neurons

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Abstract: Gamma oscillations (25-100 Hz) are thought to coordinate activity within brain regions. Two frequency bands, slow (25-50 Hz) and fast (65-90 Hz) gamma, have been

identified in the CA1 region of the rodent hippocampus. Slow gamma is phase-locked to slow gamma activity in area CA3 and presumably driven by the Schaffer Collaterals (SC), whereas fast gamma is phase-locked to fast gamma activity in the medial entorhinal cortex and presumably driven by the Perforant Path (PP). We used a combination of computational modeling and *in vitro* electrophysiology to test the hypothesis that CA1 pyramidal neurons preferentially respond to slow gamma in response to SC input activation and to fast gamma in response to PP input activation. Repetitive activation of proximal synapses of a CA1 model neuron (to mimic SC input) elicited firing in the slow gamma range, whether the stimulation was at slow or fast gamma. Action potentials were generated after each pulse in a ten-pulse, 40 Hz-train, but only after every other pulse for 100 Hz-trains. Activation of distal synapses to mimic PP input, however, elicited a single action potential for 100 Hz-trains, but not for 40 Hz. Whole-cell patch clamp recordings from CA1 pyramidal neurons in hippocampal slices *in vitro* showed that trains of 10 stimuli resulted in 8.1 ± 0.2 action potentials when SC fibers were stimulated at 40 Hz, but only 5.8 ± 0.2 at 100 Hz (n = 31), indicating a tendency to reliably follow slow, not fast gamma. This low-pass filtering tendency was greatly reduced in the presence of apamin, which blocks small conductance Ca^{2+}-activated K^{+} (SK) channels. In 10 neurons, the number of action potentials generated by trains of 10 stimuli at 100 Hz increased from 5.6 ± 0.4 under control conditions to 7.6 ± 0.5 in the presence of apamin (100 nM). On the other hand, when electrical stimulation was delivered to PP fibers, 100 Hz-trains were more effective at generating spikes than 40 Hz-trains (1.2 ± 0.2 and 0.3 ± 0.1 action potentials evoked per 10 stimuli at 100 Hz and 40 Hz, respectively; n = 11). Perfusion of the NMDA receptor antagonist APV (50 μM) decreased the number of action potentials generated in response to stimulation at these distal inputs to 0.4 ± 0.2 for 100 Hz-trains. These results suggest that CA1 pyramidal neurons might behave as low-pass filters in response to Schaffer Collateral activation and high-pass filters in response to Perforant Path activation, due to a combination of intrinsic and synaptic mechanisms.

Disclosures: C.L. Combe: None. **R. Tikidji-Hamburyan:** None. **C.C. Canavier:** None. **S. Gasparini:** None.

Poster

037. Synaptic Organization in Hippocampus

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 37.05/B64

Topic: B.07. Synaptic Transmission

Support: CIHR

Title: Regulation of entorhinal cortical input to hippocampal granule cells by local inhibitory network in the dentate gyrus

Authors: *Y. MIRCHEVA, K. TOTH;
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Abstract: Interneurons in the molecular layer of the dentate gyrus play a crucial role in the integration of entorhinal cortex (EC) input to the hippocampus. EC inputs will trigger complex network responses via the activation of various types of locally interconnected neurons such as fast-spiking basket cells and neurogliaform cells. The resulting network response will shape the firing pattern of granule cells via fast and slow inhibitory responses. In this study we aimed to determine the effect of perforant path (PP) stimulation on the firing pattern of dentate granule cells (GC). Our results show that activation of PP inputs can trigger a prolonged inhibitory response that can abolish granule cell firing for duration of 1-2 seconds. This mechanism may be involved in shaping the preferential "burst" firing pattern of GCs, information transfer at the EC-dentate gyrus gate as well as responses during periods of hyperactivity. Given the importance of this slow inhibition in information transfer and processing, we investigated the detailed properties of PP-evoked slow hyperpolarization and its physiological importance. In *in vitro* whole cell patch clamp recordings from GCs, stimulation of the perforant path with single or multiple (1 - 5) pulses evoked slow IPSPs (from ~958 ms ±34 to ~1732 ms ±182, n=10) with the potential to alter their firing pattern for an exceptionally extended period of time. This hyperpolarization was dependent on the activation of postsynaptic GABAA, GABAB and mGluR 1 receptors. Longest lasting responses were evoked when PP input was stimulated at 200 Hz. This long lasting response is specific for the feed-forward inhibitory circuit as similar response could not be evoked with the stimulation of feed-back inhibitory cells. This long lasting hyperpolarization developed in an age-dependent manner. In animals from 3 age groups (15-20 days old, 21-30 days old, 31 days and older) showed significant differences in the lengths and the amplitude of PP-evoked responses (15-20 days: mean length = 393 ms ±67.9, mean amplitude = 1.9 mV ±0.2, n = 6; 21-30 days: mean length = 1623 ms ±212, mean amplitude = 4.5 mV ± 0.7, n=6 ; 31 days and older: mean length = 2047 ms ±68, mean amplitude = 7.3mV ±0.9, n = 4). These results demonstrate the presence of an exceptionally slow IPSP in GCs in response to short train stimulation of the PP that is evolving with age. It specifically involves the feed-forward inhibitory circuit. Further investigation of the mechanism and the origin as well as the relevance for information transfer and processing of this prolonged inhibitory response, will improve our current understanding of the complex interactions at the EC-dentate gyrus gateway.

Disclosures: Y. Mircheva: None. **K. Toth:** None.

Poster

037. Synaptic Organization in Hippocampus

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Topic: B.07. Synaptic Transmission

Title: Knockout of CRMP-1 diminished intracellular calcium signal and decreased colocalization of synaptophysin and PSD-95 in primary hippocampal neurons

Authors: *Y.-C. TSAI[1], S.-R. LIN[2], T.-Y. CHIN[2], S.-M. HUANG[1];
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Abstract: Collapsing response mediator protein-1 (CRMP-1) belongs to a family of cytosolic phosphorproteins that mediate the extracellular signals from semaphorin 3A. It was initially identified in brain and has been implicated in plexin-dependent neuronal function. In order to study the physiological function of CRMP-1 in hippocampus, primary cultured hippocampal neurons were prepared from wild-type (WT) and crmp-1 knockout (KO) neonatal mice respectively. The variation in the intracellular calcium concentration ([Ca2+]i) of cultured cell was measured using Fura-2, and colocalization of pre- and post-synaptic markers was measured by immunostaining. In the studies of pre-synaptic functions, KCl caused the similar [Ca2+]i at the initial, transient stage both in WT and crmp-1 KO neurons, but at 150 seconds, crmp-1 KO neurons exhibit a higher [Ca2+]i than WT neurons. This result indicate the activation of voltage-sensitive Ca2+ channel in the initiation stage is similar in both genotypes, but after a few seconds, crmp-1 KO neurons still maintain in a higher activation of voltage-sensitive Ca2+ channel. In the studies of post-synaptic functions, glutamate caused a higher [Ca2+]i was detected in WT neurons than in crmp-1 KO neurons. On the other hand, the colocalizaton of synaptophysin and PSD-95 was decreased in crmp-1 KO neurons. Our results implied knockout of CRMP-1 influenced the pre- and post-synaptic function, and even decreased the synaptogenesis. However, the mechanisms for explaining the difference phenotype between WT and crmp-1 KO neurons need further investigations.

Disclosures: **Y. Tsai:** None. **S. Lin:** None. **T. Chin:** None. **S. Huang:** None.

Poster

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Topic: B.07. Synaptic Transmission

Support: NSERC Grant 2014-05407

FRQ-S Groupe de Recherche

Title: The parasubiculum heterosynaptically modulates the strength of piriform cortex inputs to the medial entorhinal cortex

Authors: *D. W. SPARKS, C. A. CHAPMAN;
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Abstract: The hippocampus receives the majority of its sensory input from the entorhinal cortex, and the entorhinal cortex receives dense sensory input from the piriform cortex. Layer II of the entorhinal cortex also receives the single major output projection of the parasubiculum, which receives projections from multiple hippocampal subfields and subcortical areas. Previous research in our lab found that stimulation of the parasubiculum can enhance entorhinal responses to piriform inputs *in vivo*, suggesting that the parasubiculum modulates sensory input to the hippocampus via the entorhinal cortex. Spatial and mnemonic processes in these brain areas are modulated by cholinergically-induced rhythmic activity, and our lab has also found that synaptic responses in the entorhinal cortex *in vitro* during trains of theta- and gamma-frequency stimulation of the parasubiculum are enhanced following application of the cholinergic agonist carbachol via effects on the non-specific cationic channel Ih. The purpose of the current study was to investigate how parasubicular inputs modulate the responsiveness of individual entorhinal cortex cells to piriform cortex stimulation *in vitro*. We also assessed if rhythmic stimulation of the parasubiculum could heterosynaptically modulate synaptic input to the entorhinal cortex from the piriform cortex, and assessed the contribution of Ih. Patch-clamp recordings from layer II medial entorhinal neurons were obtained in slices from four to eight-week old male Long-Evans rats, and bipolar tungsten stimulating electrodes were placed in the parasubiculum and piriform cortex. Single pulses and trains of stimulation at theta-frequency were delivered to the parasubiculum, followed by single pulses to the piriform cortex at various intervals. Consistent with previous *in vivo* findings, parasubicular stimulation enhanced responses to piriform cortex stimulation at a 25ms interval. This effect, however, was only found following theta-frequency trains of parasubicular stimulation, as opposed to single pulses. This suggests that rhythmic depolarizing drive from the parasubiculum can enhance incoming sensory input to the entorhinal cortex. Bath application of the Ih blocker ZD7288 caused an enhancement of this effect, suggesting that cholinergic inhibition of Ih may facilitate heterosynaptic modulation of piriform inputs to the entorhinal cortex by the parasubiculum. These findings provide further evidence that theta activity in the entorhinal cortex is associated with short-term heterosynaptic facilitation effects that may play a role in the parasubicular modulation of sensory inputs to the entorhinal cortex.

Disclosures: **D.W. Sparks:** None. **C.A. Chapman:** None.

Poster

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Support: CIHR

NSERC

NSERC Postgraduate Scholarship (to OC)

Title: Synaptic integration gradients in dendrites of hippocampal CA1 interneurons reflect heterogeneity of the local excitatory input

Authors: ***O. CAMIRÉ**[1,2], I. LAZAREVICH[3], V. KAZANTSEV[3], L. TOPOLNIK[2]; [1]IUSMQ, Quebec, QC, Canada; [2]Biochemistry, Microbiology and Bio-informatics, Univ. Laval, Québec City, QC, Canada; [3]Nizhny Novgorod State Univ., Nizhny Novgorod, Russian Federation

Abstract: Two structurally and functionally distinct subtypes of pyramidal neurons(superficial and deep PYRs) have been identified along the radial axis of the PYR layer in the hippocampal CA1 region. These cells can be further distinguished by differential recruitment of CA1 inhibitory interneurons. But the mechanisms that control the integration of inputs from different PYRs in interneuron dendrites remain unknown. Here, we addressed this question using a combination of two-photon microscopy, whole-cell patch-clamp recordings and computational simulations in basal dendrites of CA1 parvalbumin-positive (PV+) basket and bistratified cells. We first examined the mechanisms of synaptic integration along a somatodendritic axis of interneurons. We found significant fluctuations in the mechanisms of postsynaptic Ca2+ influx and in summation of Ca2+ signals at excitatory synapses formed on PV+ cells by CA1 PYRs, indicative of the synapse-specific dendritic integration. Analysis of computational simulations showed that, as a rule, the activation of 7 to 8 synapses was sufficient for the initiation of dendritic Ca2+ nonlinearities, which at distal synapses relied mostly on Ca2+ influx through activation of Ca2+ -permeable AMPA receptors and Ca2+ release from the internal stores. However, a higher NMDA:AMPA receptor ratio detected experimentally at more proximal synapses slowed the decay of Ca2+ nonlinearities with a direct impact on somatic input. In

particular, a higher slope of the voltage derivative and, accordingly, a larger number of action potentials was associated with Ca2+ nonlinearities induced at synapses with higher NMDA receptor content, leading to the interneuron bursting. As these synapses could be primarily formed by the superficial PYRs, the latter may play a preferential role in driving the interneuron recruitment. These data indicate that heterogeneity of hippocampal PYRs may be encoded in interneuron dendrites, thus determining their input-specific recruitment and firing behaviour during different brain states.

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Poster

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Title: Increased dosage of high affinity kainate receptor gene grik4 alters synaptic transmission and produces autism spectrum disorders features

Authors: *M. I. ALLER, V. PECORARO, A. V. PATERNAIN, S. CANALS, J. LERMA; Inst. de Neurociencias, San Juan de Alicante, Spain

Abstract: The GRIK4 gene, coding for the high affinity kainate receptor (KAR) subunit GluK4, has been associated with several mental disorders and intellectual disability. This gene maps in the human chromosomal band 11q23.3. This region seems to be an instable region that in some cases may undergo translocation. For instance, the Enmanuel syndrome is characterized by a duplication of this region which translocate in the chromosome 22, generating a complex set of symptoms, including intellectual disability. Although not definitively documented, the GRIK4 gene has been involved in mental diseases such as depression, bipolar disorders, and more recently a duplication of this gene has been found in a patient of autism. KARs play an important role in synaptic transmission, particularly in the hippocampus. To understand which are the physiological consequences of a gain of function of this gene, we developed a new transgenic mouse overexpressing the GluK4 subunit in the forebrain. To this end, the expression

of exogenous grik4 tagged with 5 myc was driven by the CaMKII promoter. These mice showed a 25% increase in the body weight. Western blot studies revealed that the transgene gave rise to the overexpression of GluK4 30-100% above the endogenous levels. Inmunocytochemical data indicated the presence of exogenous GluK4 in the hippocampus, especially in CA3 and DG regions, that normally express this subunit, at pre and postsynaptic sites. Spontaneous EPSCKAR recorded in CA3 pyramidal cells presented 38% larger amplitude than in normal mice and faster deactivation times, indicating that exogenous protein was correctly inserted at synaptic sites. Surprisingly, AMPA receptor-mediated mEPSC had both larger frequency and amplitude in the GluK4over mice. Behavioral studies in these mice revealed high levels of anxiety, less social interaction, anhedonia and depression, similar symptoms as can be found in autism and depressive patients.. By using *in vivo* electrophysiology we observed augmented information transfer through the trisynaptic hippocampal circuit, which was indicative of altered synaptic transmission. In conclusion these data show that increased levels of GluK4 subunits alter synaptic transmission and generates behavioral symptoms compatible with those seen in autism spectrum disorders, demonstrating a clear link of KARs with mood disorders and revealing new targets for their treatments.

Disclosures: M.I. Aller: None. **V. Pecoraro:** None. **A.V. Paternain:** None. **S. Canals:** None. **J. Lerma:** None.

Poster

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Topic: B.07. Synaptic Transmission

Support: NIMHF32100745 (LYC)

NIMH R37 MH052804 (TCS)

Title: Role of neurexin 2 (nrxn2) in cortex and hippocampus: synaptic analysis of conditional nrxn2 knockout mice

Authors: *L. Y. CHEN, P. ZHOU, A. ORTIZ, T.-L. HUYNH-TRAN, T. C. SUDHOF; Stanford Inst. for Neuro-innovation & Translational Neurosciences, Stanford Univ., Stanford, CA

Abstract: Increasing evidence suggests that neurexins (Nrxns) are presynaptic cell adhesion molecules that are critical for maintaining synaptic function. NRXN genes have been linked to autism spectrum disorders (ASD) and schizophrenia. In particular, recent studies identified patients with ASDs that have a truncating mutation of NRXN2. However, due to the lacking of fundamental understanding of Nrxn2 function, it remains unknown why Nrxn2 is associated with these disorders. Here, we made, characterized, and used conditional knockout (cKO) mice in which both the α- and β-forms of NRXN2 can be deleted by Cre recombinase. These mice allow us to investigate the synaptic function and requirements for NRXN2. The design and creation of this cKO NRXN2 mouse was confirmed by genotyping and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) to measure mRNA levels. We tested our hypothesis that Nrxn2 is required for normal synaptic function and synapse formation in both hippocampus and cortex. Analysis of spontaneous miniature postsynaptic currents, evoked responses and synaptic morphology of Cre-induced knockout neurons (or with overexpression of specific Nrxn2 isoform for rescue) in comparison with control neurons (a mutated version of Cre: ΔCre). This study will address the precise contributions of specific NRXN isoforms to synaptic transmission and identify pathways relevant to synapse development that give rise to cognition and brain function.

Disclosures: L.Y. Chen: None. **P. Zhou:** None. **A. Ortiz:** None. **T. Huynh-Tran:** None. **T.C. Sudhof:** None.

Poster

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Topic: B.07. Synaptic Transmission

Title: Input-specific synaptic differences in hippocampal CA1 parvalbumin-positive fast-spiking basket cells

Authors: *J. CORNFORD, D. KULLMANN;
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Abstract: Although PV+ interneurons make up <3% of interneurons in the CA1 region of the hippocampus, they are fundamental for a range of functions, from basic circuit processes to more complex network operations such as place cell field regulation (Hu et al., 2014). The importance of PV+ basket cells is partly explained by their specialised input co-incidence detection mechanisms, which allow them to convert asynchronous input into precisely timed output (Bartos & Elgueta, 2012). Recent work has suggested that basket cells express synaptic receptors

in an input specific distribution: local feedback excitatory synapses onto oriens dendrites display a much larger NMDA receptor component than synapses in the stratum radiatum, which are dominated by calcium permeable (CP) AMPA receptor currents (Le Roux et al., 2013). This raises the possibility that PV+ basket cells integrate afferent activity in different ways, as in contrast to radiatum inputs, synaptic connections from local pyramidal cells could co-operate via NMDA receptors (Branco & Häusser, 2011). In order to investigate the distinct expression of rectifying glutamate receptors and its functional implications, we used a combination of neuronal modelling, multi-photon imaging, and glutamate uncaging. We first looked for structural differences between basket cell dendrites in the CA1 stratum oriens and the stratum radiatum. We found that dendrites in the oriens express spines in a sparse distribution, whereas dendrites in the radiatum are smooth. Hypothesising that these spines might be the location of postsynaptic sites containing both NMDA and AMPA receptors, we modelled how dendrites expressing synapses with different properties might behave when presented with various input patterns. We show that CP-AMPA receptors enhance the detection of input coincidence, whereas NMDA receptors promote input co-operation. Further experiments employing glutamate uncaging onto smooth dendrites in stratum radiatum, and spiny dendrites in stratum oriens, will seek to further clarify differential input processing mechanisms by fast spiking PV+ basket cells. Bartos, M. & Elgueta, C. (2012) J. Physiol. (Lond.). Branco, T. & Häusser, M. (2011) Neuron, 69, 885-892. Hu, H., Gan, J., & Jonas, P. (2014) I Science, 345, 1255263. Le Roux, N., Cabezas, C., Böhm, U.L., & Poncer, J.C. (2013) J. Physiol. (Lond.), 591, 1809-1822.

Disclosures: J. Cornford: None. **D. Kullmann:** None.

Poster

037. Synaptic Organization in Hippocampus

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Topic: B.08. Synaptic Plasticity

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Title: Gap junction channels in the hippocampal plasticity in an *in vivo* model of high neuronal activation

Authors: *E. R. KINJO[1], B. A. SANTOS[1], G. S. V. HIGA[1,3], M. M. G. AGUIAR[1], É. SOUSA[2], A. H. KIHARA[1];
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Abstract: Gap junction channels (GJC) are intercellular conduits that enable the exchange of small molecules such as second messengers and ions between two adjacent cells, allowing the occurrence of electrical synapses. These channels are composed by protein subunits called connexins (Cx). So far, at least 20 Cx genes were described in human and mouse genome, and Cx36 and Cx45 were identified in neuronal cells. The involvement of these channels in the synchronization of neuronal oscillations has been increasingly demonstrated, which in turn are important for processes regarding synaptic plasticity. While most efforts have been dedicated to the understanding of chemical synapses, less is known regarding the plastic properties of electrical synapses. By using an *in vivo* model of neuronal activation induced by pilocarpine, our goal was to evaluate the expression of the neuronal Cxs 36 and 45 in the hippocampus of rats combining real time PCR and western blot analysis. Male wistar rats were previously treated with methyl scopolamine (1 mg/kg; subcutaneous) followed by intraperitoneal pilocarpine (360 mg/kg) injection. Control animals received saline instead of pilocarpine. Thirty minutes after the establishment of status epilepticus, the animals were sacrificed and the hippocampi removed for real time PCR and western blot analysis. Pilocarpine application promptly induced changes in rat electrocorticogram, particularly in fast-beta range (21-30 Hz), demonstrating high neuronal activation. Real time PCR analysis showed no significant changes in Cx36 mRNA levels after neuronal activation, while increase in Cx45 gene expression ($p<0.01$) was detected. The western blot analysis revealed no significant alterations in Cx36 protein levels, in contrast to an increase of 23% ($p<0.05$) in Cx45 protein levels in the hippocampi of animals submitted to intense neuronal activation. These data suggest modulation of GJC in the activity-dependent plasticity, favoring the idea that electrical synapses can be modulated and play important role in shaping the neuronal electrical activity.

Disclosures: E.R. Kinjo: None. **B.A. Santos:** None. **G.S.V. Higa:** None. **M.M.G. Aguiar:** None. **É. Sousa:** None. **A.H. Kihara:** None.

Poster

038. Short Term Plasticity

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Topic: B.08. Synaptic Plasticity

Support: the Brain Korea 21 PLUS Program

Title: Short-term potentiation of dendritic Na^+ spikes and action potentials after high frequency stimulation at Schaffer collateral-CA1 synapses

Authors: W. YU[1], J. KWON[1], J.-W. SOHN[2], S.-H. LEE[1], *W.-K. HO[1];
[1]Seoul Natl. Univ. Col. Med., Seoul, Korea, Republic of; [2]KAIST, Daejeon, Korea, Republic of

Abstract: Active properties of dendrites are important in the process of how local synaptic inputs are integrated and transmitted to the soma to generate propagating action potentials. In particular, Na^+-dependent dendritic spikes play crucial roles in the amplification of synaptic potentials to increase the efficacy of axosomatic action potential initiation and modulation of synaptic plasticity. In this study, we demonstrated that after high frequency stimulation (HFS, 50 stimuli at 100 Hz) to the Shaffer collateral pathway near proximal apical dendrites of pyramidal cells in the CA1 hippocampus (CA1-PCs) EPSP-spike coupling was potentiated significantly (E-S potentiation), which lasted for about 2 min. Dendritic patch recordings revealed that probability of the generation of dendritic spikes was increased significantly after HFS. We further investigated ion channel mechanism and found that inhibition of persistent Na^+ current ($I_{Na,P}$) using riluzole abolished E-S potentiation and increased dendritic spikes after HFS. As underlying signaling mechanisms, we found that E-S potentiation was abolished by inhibiting mGluR5 (MPEP or mGluR5 knock-out) or inhibiting mGluR5-dependent Ca^{2+} release using cADP ribose blockers (8-NH2-cADPR and nicotinamide) or ryanodine. Voltage clamp analysis confirmed that group I mGluR agonist, DHPG, increased $I_{Na,P}$, especially when DHPG was applied locally to the apical dendrites, and that DHPG-induced $I_{Na,P}$ potentiation was abolished by inhibiting mGluR5-dependent Ca^{2+} release or calmodulin. These results provide a novel insight into how the activity-dependent regulation of dendritic ion channels by metabotropic receptor signaling leads to the regulation of action potential outputs.

Disclosures: W. Yu: None. **J. Kwon:** None. **J. Sohn:** None. **S. Lee:** None. **W. Ho:** None.

Poster

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Support: CIHR

CRC

Title: Determinants of synaptic heterogeneity at the mature calyx of Held synapse

Authors: *A. FEKETE, L.-Y. WANG;
Program in Neurosci. and Mental Hlth., The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: The heterogeneity of release probability (Pr) and short-term plasticity (STP) among synapses on the same population of neurons is a fundamental feature of the central nervous system, and thought to be important for underlying different dynamic range of information coding. However, the origin of synaptic heterogeneity remains largely unknown. We addressed this issue by taking advantage of the large size of the mouse calyx of Held synapse, where the morphological variability strongly correlates with, and predicts the differences in Pr, polarity of STP and fidelity of spiking (Grande and Wang, 2011). We have examined single and high-frequency train stimuli-evoked Ca2+ dynamics mediated solely by P/Q-type Ca2+ channels in distinct compartments of different types of calyces with two-photon laser scanning microscopy of high- and low-affinity Ca2+-indicators and the morphological tracer Alexa 594. We found Ca2+ transients throughout the entire calyceal arborization, being the largest in the smallest compartments (swellings). Application of potassium channel blocker TEA increased the Ca2+ transients throughout the calyx, suggesting that APs can successfully propagate into distinct compartments and recruit increasing number of calcium channels to boost Ca2+ transients. AP propagation into small and large compartments was independently validated by direct cell-attached recordings at different sites of the same calyx. Patch-clamp recordings showed a significantly higher Ca2+ channel density in complex calyces than that in simple ones. Presynaptic injections of slow Ca2+ buffer EGTA attenuated Pr and RRP more robustly in complex calyces. Our data implicate differences in the density of Ca2+ channels and their spatial coupling distance to synaptic vesicles as key elements underpinning the heterogeneity of presynaptic Ca2+ transients and quantal parameters, ultimately leading to functional diversity of central synapses.

Disclosures: **A. Fekete:** None. **L. Wang:** None.

Poster

038. Short Term Plasticity

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Topic: B.08. Synaptic Plasticity

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Title: Influence of a single session of maximal intensity aerobic exercise on the post-translational modification and trafficking of plasticity-related receptor proteins within the motor cortex

Authors: *J. S. THACKER[1], W. R. STAINES[1], J. G. MIELKE[2,1];
[1]Kinesiology, [2]Sch. of Publ. Hlth. & Hlth. Systems, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: A substantial body of research has focused on developing techniques that enhance the recovery of motor function after injury by priming neural plasticity. A recent focus in the area has involved using multiple sessions of aerobic exercise as a variety of evidence has indicated that exercise can stimulate the expression of proteins thought to play a role in plasticity. For example, the interaction of brain derived neurotrophic factor (BDNF) and its receptor tropomyosin kinase B (TrkB), which have been linked to various features of plasticity, are clearly affected by aerobic training. Notably, while the effects of multiple bouts of exercise training are becoming established, comparatively less is known about the effects that may result from a single session of exercise. As a result, the aim of the current investigation was to explore the influence that a single session of maximal intensity aerobic exercise could have on a set of proteins previously identified as being both influenced by exercise and important for plasticity. We hypothesized that a single session of maximal aerobic exercise would lead to changes in BDNF-TrkB signal transduction, as well as altered trafficking of glutamate receptor proteins, in the motor cortex. Young (post-natal day 60), male Sprague-Dawley rats (n = 20) on a reverse light cycle (12 h/12 h) were exposed to a treadmill acclimatization procedure that consisted of 8 days of increasing exercise intensity (speed = 10 m/min up to 25 m/min) for 10 minutes at the same time (10 am) each day. The acclimatization period was followed by 2 days of rest to reduce the possibility of carryover effects from the treadmill training. On testing day, rats were separated into either a maximal intensity aerobic exercise group (VO2 max test), or a non-exercising group. Immediately following the bout of maximal intensity aerobic exercise (or a sedentary period of a similar length), rats were sacrificed and brains were dissected into varying regions (motor cortex, prefrontal cortex, cerebellum, and brainstem) and either homogenized, or flash frozen at -80°C. Brain homogenates were probed for all proteins of interest using Western blotting. Preliminary results suggest no significant differences in either the expression, or phosphorylation level (Y813) of TrkB receptor in motor cortex following a single session of intense aerobic exercise. However, further analysis of other plasticity-related proteins, including Calcium-calmodulin kinase II, insulin-like growth factor and its receptor, as well as the synaptic trafficking of NMDA and AMPA receptors, remain to be explored.

Disclosures: **J.S. Thacker:** None. **W.R. Staines:** None. **J.G. Mielke:** None.

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Topic: B.08. Synaptic Plasticity

Support: NIMH Grant R15 MH092866-01A1

Title: Effects of dopaminergic D2 receptor activation on layer I and layer V evoked excitatory synaptic responses in mouse medial prefrontal cortex

Authors: *J. M. LEYRER[1], M. P. THOMAS[2];
[1]Univ. Of Northern Colorado, Greeley, CO; [2]Sch. of Biol. Sci., Univ. of Northern Colorado, Greeley, CO

Abstract: In humans, prefrontal cortical areas are known to support executive functions. In mice, these functions are mediated by homologous regions in the medial prefrontal cortex (mPFC). While it is well established that executive processes are critically dependent on optimal levels of dopamine (DA) in the PFC, the cellular mechanisms of DA modulation are incompletely understood. Stable patterns of neuronal activity may be sensitive to frequency dependent changes in inhibitory and excitatory transmission. In this study, we characterized the effects of D2 receptor (D2R) activation on short-term excitatory postsynaptic potential (EPSP) dynamics evoked at varying frequencies (10Hz-50Hz) in layer V pyramidal neurons in mouse mPFC. We isolated NMDA receptor (NMDAR) and non-NMDAR receptor mediated components of EPSP trains evoked by stimulating fibers within layer V or layer I (tufts). D2R activation had no effect on non-NMDAR mediated EPSPs with layer V or layer I stimulation, while decreasing the amplitude of NMDAR mediated EPSP trains with both layer V and layer I stimulation. These results suggest that D2R activation acts by restricting synaptic plasticity at both layer V and layer I excitatory synapses, stabilizing existing connectivity patterns. Our previous studies demonstrate that D1R and D2R activation have similar effects on layer I excitatory synapses. However, with layer V stimulation, D1R activation enhanced both NMDA and non-NMDA EPSPs, which suggests that when D1R activation predominates, plasticity is promoted; and when D2R activation predominates, plasticity is suppressed. These data provide further insight into mechanisms of dopamine's bidirectional modulation of executive functions.

Disclosures: J.M. Leyrer: None. **M.P. Thomas:** None.

Poster

038. Short Term Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 38.05/B76

Topic: B.08. Synaptic Plasticity

Support: NIH grant EY-12782 to MJF

Title: Effects of irregular (Poisson) conditioning trains on synaptic plasticity between individual neurons in layer 4 of mouse visual cortex

Authors: *J. WU, M. J. FRIEDLANDER;
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Abstract: Mammalian primary visual cortex Layer 4 (L4) neurons receive monosynaptic excitatory input from the dorsal lateral geniculate nucleus, from layer 6 and from adjacent L4 cells. Synaptic integration with layer 4 has considerable effect on visual information processing and has been hypothesized to act as an amplifier of thalamocortical inputs. Though the thalamocortical pathway has been extensively studied, less is known about the functional properties and synaptic plasticity between individual L4 neurons. To better understand plasticity of synaptic connections between individual L4 neurons, and the effect of different conditioning patterns on plasticity, we performed dual and triple whole cell patch clamp recording from L4-L4 pairs (n=17) in visual cortical slice of P25~35 C57B1/6 mice. A pair of action potential was elicited in the L4 presynaptic neuron with a pair of square depolarizing pulses (5ms duration, 600~800pA, 30ms inter spike) at 0.1Hz, the unitary postsynaptic currents were recorded from the postsynaptic neuron under voltage clamp. In some case (n=6), a 15min 10Hz conditioning protocol was applied with a Poisson distribution of interstimulus intervals having a coefficient of variation (CV) = 1.0 to induce synaptic plasticity. The synaptic strength, potency, failure rate and paired pulse ratio were calculated before and after conditioning. The pre-conditioning control L4 baseline synaptic strength varied from 0.64 to 71.6 pA with an average of 19.2 ±3.7pA (n=17). 13 pairs of connection were reliable, the failure rate ≤ 30% and the other 4 unreliable pairs failure rate >50%. The low failure rate is correlated with paired pulse depression (PPD), while the high failure rate is correlated with paired pulse facilitation (PPF). Short term plasticity (PPD and PPF) was heterogeneous - 9 pairs of connection exhibited PPD (0.69±0.04), 6 pairs exhibited PPF (1.61±0.25) and 2 pairs exhibited no short term plasticity (1.01±0.01). After conditioning, net synaptic strength decreased (7.4±2.8 *vs* 12.0±4.2 pA, $p<0.05$, paired t-test) and failure rate increased (35.3±15.7 *vs* 16.5±5.8%, n=6); one pair switched from PPF (1.40) to PPD (0.67); one pair remained unchanged; in the remaining 4 pairs, PPD increased (0.81±0.4 *vs* 0.65±0.32

,p<0.01 paired t-test). Among the 6 pairs that underwent 10 Hz Poisson distributed conditioning, 3 underwent LTD (0.40 ±0.13, p<0.05), and one underwent LTP (1.28, p<0.01, KS-test).

Disclosures: J. Wu: None. **M.J. Friedlander:** None.

Poster

038. Short Term Plasticity

Location: Hall A

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Program#/Poster#: 38.06/B77

Topic: B.08. Synaptic Plasticity

Support: Biotechnology and Biological Sciences Research Council (grant: BB/F008953/1

Berand Neuropharmacology

Title: A role for hippocampal PSA-NCAM and NMDA-NR2B receptor in flavonoid-induced spatial memory improvements in young rats

Authors: *C. RENDEIRO[1], A. FOLEY[2], C. M. WILLIAMS[3], C. REGAN[2], J. P. E. SPENCER[3];
[1]Beckman Inst., Univ. of Illinois, Urbana, IL; [2]Berand Neuropharmacology, NovaUCD, Belfield Innovation Park, UCD, Belfield, Dublin, Ireland; [3]Univ. of Reading, Reading, United Kingdom

Abstract: The increase in incidence and prevalence of neurodegenerative diseases highlights the need for a more comprehensive understanding of how food components may affect neural systems. In particular, flavonoids have been recognized as promising agents capable of influencing different aspects of synaptic plasticity resulting in improvements in memory and learning in both animals and humans. Our previous studies highlight the efficacy of flavonoids in reversing memory impairments in aged rats, yet little is known about the effects of these compounds in healthy animals, particularly with respect to the molecular mechanisms by which flavonoids might alter the underlying synaptic modifications responsible for behavioral changes. We demonstrate that a 3-week intervention with two dietary doses of flavonoids (Dose I: 8.7 mg/day and Dose II: 17.4 mg/day) facilitates spatial memory acquisition and recall (24 h) ($p < 0.05$) in the Morris Water Maze in young healthy rats. We show for the first time that these behavioral improvements are linked to increased levels in the polysialylated form of the neural adhesion molecule (PSA-NCAM) in the dentate gyrus (DG) of the hippocampus, which is known to be required for the establishment of durable memories. We observed parallel increases in hippocampal NMDA receptors containing the NR2B subunit for both 8.7 mg/day ($p < 0.05$) and

17.4 mg/day ($p < 0.001$) doses, suggesting an enhancement of glutamate signaling following flavonoid intervention. This is further strengthened by the simultaneous modulation of hippocampal ERK/CREB/BDNF signaling and the activation of the Akt/mTOR/Arc pathway which are crucial in inducing changes in the strength of hippocampal synaptic connections that underlie learning. Through these mechanisms, the consumption of flavonoid-rich foods throughout life holds the potential to limit neurodegeneration and to prevent or reverse age-dependent loses in cognitive performance.

Disclosures: **C. Rendeiro:** None. **A. Foley:** None. **C.M. Williams:** None. **C. Regan:** None. **J.P.E. Spencer:** None.

Poster

038. Short Term Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 38.07/B78

Topic: B.08. Synaptic Plasticity

Support: KAKENHI Grant 24300144

KAKENHI Grant 24650218

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JSPS/MEXT Core-to-Core Program A Advanced Research Networks

Toray Science Foundation

Uehara Foundation

Title: Short-term facilitation is predominantly mediated by presynaptic Ca^{2+} current facilitation at Purkinje cell - Purkinje cell synapses

Authors: *F. DÍAZ-ROJAS, T. SAKABA, S.-Y. KAWAGUCHI;
Doshisha Univ., Kyotanabe, Japan

Abstract: Short-term facilitation (STF) of synaptic transmission plays a critical role in neural information processing. Several hypotheses have been proposed to explain this process: temporal summation of residual Ca^{2+}, modulation of action potential (AP) waveform, saturation of Ca^{2+}

buffer protein, facilitation of Ca^{2+} current (I_{Ca}^{2+}) through voltage-gated Ca^{2+} channels, and Ca^{2+}-dependent positive modulation of vesicle release machinery. We attempted to clarify the mechanism mediating STF at GABAergic synapses between cerebellar Purkinje cells (PCs) using dissociated rat cerebellar cultures. We performed fluorescent imaging of residual Ca^{2+} increase upon an action potential in PC axon terminals using OGB-1 or OGB-6. PC terminals exhibited only a tiny increase in the fluorescence intensity upon stimulation single AP. With this information, we estimated the residual Ca^{2+} concentration, which could explain only 1% of the facilitation. Alternatively, direct patch-clamp recording from a PC axon terminal showed that presynaptic I_{Ca}^{2+} was facilitated depending on the intracellular Ca^{2+} concentration upon paired pulses of AP-like waveforms. Application of BAPTA but not EGTA suppressed the facilitation of I_{Ca}^{2+} suggesting that this facilitation is tightly coupled with Ca^{2+} influx. Taking it into consideration that synaptic transmission shows about 4^{th} power dependency on the presynaptic Ca^{2+} concentration, the I_{Ca}^{2+} facilitation almost completely explained the facilitation of IPSC at PC-PC synapses. Importantly, suppression of the I_{Ca}^{2+} facilitation by modulation of the AP waveforms eliminated the facilitation of IPSCs. Our data suggests that the Ca^{2+}-dependent facilitation of the presynaptic I_{Ca}^{2+} fully accounts for the facilitation observed at the PC-PC synapse.

Disclosures: **F. Díaz-Rojas:** None. **T. Sakaba:** None. **S. Kawaguchi:** None.

Poster

038. Short Term Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 38.08/B79

Topic: B.08. Synaptic Plasticity

Support: JSPS KAKENHI #24700406

Title: Acute presynaptic loading of α-syunclein impairs synaptic fidelity by slowing vesicle endocytosis at glutamatergic synapses

Authors: ***K. EGUCHI**, Z. TAOUFIQ, T. TAKAHASHI;
Okinawa Inst. of Sci. and Technol. Grad. Univ., Onna-Son, Kunigami, Okinawa, Japan

Abstract: α-Synuclein is a small protein highly localized in presynaptic terminals. In sporadic and familial Parkinson's diseases (PDs), its expression is found to be elevated by several-fold. To address how the elevation of α-synuclein can influence neurotransmission, we loaded recombinant human α-syunclein directly into the calyx of Held presynaptic terminals in

brainstem slices of rats, using whole-cell patch-clamp method.α-Synuclein loaded at 3.6 μM had no effect on basal synaptic transmission, but slowed the recovery of EPSCs from synaptic depression, and impaired the fidelity of neurotransmission during high frequency stimulation. Membrane capacitance measurements from presynaptic terminals indicated that α-synuclein slowed endocytosis of synaptic vesicles with no effect on calcium currents or vesicle exocytosis. In contrast to wild-type α-synuclein, its A30P mutant had no significant effect on the endocytic rate. Strikingly, co-loading with α-synuclein of the microtubules depolymerizing drug nocodazole rescued the inhibitory effects of α-synuclein on vesicle endocytosis, recovery rate of EPSCs after synaptic depression, and synaptic fidelity. We conclude that excess α-synuclein primarily targets vesicle endocytosis and impairs precision of fast neurotransmission via aberrant assembly of microtubules in the mammalian glutamatergic nerve terminals.

Disclosures: K. Eguchi: None. **Z. Taoufiq:** None. **T. Takahashi:** None.

Poster

038. Short Term Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: B.08. Synaptic Plasticity

Support: Operating Grant from CIHR

University of Calgary

Eyes High Postdoctoral Program

AIHS

Title: Stress contagions at glutamate synapses in the paraventricular nucleus of the hypothalamus

Authors: *T.-L. STERLEY, D. BAIMOUK, J. BAINS;
Univ. of Calgary, Calgary, AB, Canada

Abstract: Experiencing a stressor causes short and long-term changes at synapses in various brain regions. We have previously shown that exposure to a single acute stress ‘primes’ glutamate synapses in the paraventricular nucleus of the hypothalamus - the brain region at the apex of the hypothalamic-pituitary-adrenal axis. This priming of glutamate synapses allows them to undergo short-term potentiation following a burst of high-frequency afferent activity. A

number of reports have shown that rodents exposed to specific stimuli can transfer information that alters the behavior of cagemates not exposed to the stimulus. Whether this transfer can be detected at the level of individual synapses is not clear. Here we housed male mice (Crh-IRES-Cre, tdTomato) in pairs and subsequently exposed one of the pair to a stressor (5 minute footshock, 0.5 mA for 2 seconds every 30 seconds). We then allowed the pair to interact for 30 minutes. During this period, the pair engaged in social interactions including sniffing and grooming as well as self-grooming. We then anaesthetized both animals and prepared coronal brain slices to conduct electrophysiological experiments in the PVN. Glutamate synapses on CRH neurons from mice exposed to footshock were potentiated is response to bursts of afferent activity (single housed: 160 ± 12.91, n = 24; with littermate: 126.28 ± 12.08, n = 14) compared to single housed naïve animals (99.66 ± 15.06, n = 11). In addition, we observed the same synaptic phenotype in the naïve conspecific littermate (141.55 ± 14.43, n = 19). This synaptic phenotype was not evident in male littermates only separated for 5 minutes without footshock (107.25 ± 7.83, n = 30). These observations demonstrate that the impact of acute stress on one animal can manifest as synaptic changes in another animal. This is, to the best of our knowledge, the first demonstration of stress contagions at the level of the synapse.

Disclosures: **T. Sterley:** None. **D. Baimouk:** None. **J. Bains:** None.

Poster

038. Short Term Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 38.10/B81

Topic: B.08. Synaptic Plasticity

Support: NIH P41-GM103712

Howard Hughes Medical Institute

Title: An event-driven model of short-term presynaptic dynamics based on detailed molecular simulations with MCell

Authors: ***J. W. GARCIA**[1], T. M. BARTOL[1], D. J. SPENCER[1], T. J. SEJNOWSKI[2];
[1]Salk Inst. for Biol. Studies, La Jolla, CA; [2]Howard Hughes Med. Inst., La Jolla, CA

Abstract: Chemical synapses enable information transmission between neurons throughout the brain and are generally considered fundamental to learning and memory. We propose a novel model designed to efficiently reproduce their short-term dynamics. In biological synapses, action

potentials trigger an influx of calcium ions through voltage-gated channels. These ions stochastically bind with the vesicular release machinery with some delay, causing probabilistic release of neurotransmitter that occurs asynchronously with incoming spikes. Nonlinear calcium binding and unbinding kinetics both determine the timing of neurotransmitter release and enable facilitation of release probability between spikes, while depletion of the finite vesicle pool in the presynaptic space depresses the release probability. Although many synaptic models implement probabilistic release and some form of short-term plasticity, most of them do not take asynchronous release into account. Detailed molecular models of the presynaptic machinery, on the other hand, achieve all these features but with huge cost in computational efficiency. To investigate the significance of these dynamics in the context of larger networks, we designed our presynaptic model to capture the phenomenology of the molecular models while maintaining the speed and efficiency of the simpler models. The model achieves both probabilistic, asynchronous release and facilitation by matching the release profiles from detailed molecular simulations in MCell, and the time course for depression is implemented with exchanges between vesicle pools. The model treats spikes, releases, and vesicle exchanges as point events, allowing it to skip over intervening time without computational overhead by asynchronously calculating the timing of future events. It thus has significant advantages in biological realism, efficiency, and scalability.

Disclosures: **J.W. Garcia:** None. **T.M. Bartol:** None. **D.J. Spencer:** None. **T.J. Sejnowski:** None.

Poster

038. Short Term Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 38.11/B82

Topic: B.08. Synaptic Plasticity

Title: Post-synaptic Pannexin-1 alters synaptic fidelity in the CA3-CA1 synapse of the hippocampus

Authors: ***J. BIALECKI**[1], N. L. WEILINGER[2], M. N. HILL[3], R. J. THOMPSON[3];
[2]Neurosci., [3]Cell Biol. and Anat., [1]Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Pannexin-1 is a large pore ion channel that is capable of passing molecules up to 1 kilodalton. It is ubiquitously found in the brain and is highly expressed in post-synaptic hippocampal pyramidal neurons. Pannexin-1 is known to play a role in pathological conditions such as stroke, where the activation of Panx1 leads to ionic dysregulation and the anoxic depolarization. However, possible physiological roles of Panx1 are not well understood. We

hypothesize that Panx1 plays a role in synaptic physiology under physiological conditions. Using whole- cell patch clamp electrophysiological recordings of CA1 pyramidal neurons and through the use of various pharmacological blockers of Panx1, we observed that blocking Panx1 leads to an up-regulation of pre-synaptic glutamate release. When post-synaptic Panx1 is blocked through the use of anti-Panx1 in the patch pipette, a large increase in sEPSP frequency was detected. Increased glutamate release is also seen with other Panx1 antagonists including ^{10}Panx, and this increase is not seen when using a scrambled version of ^{10}Panx. Interestingly, increased glutamate in the presence of anti-Panx1 or ^{10}Panx does not occur under baseline recording conditions, but required stimulation of the Schaffer Collaterals. Various stimulation paradigms were assessed to determine the intensity and frequency of stimulation necessary to excite the synapse. We are now working towards the synaptic mechanism by which Panx1 is able to alter pre-synaptic transmission. Retrograde transmitters such as 2-AG and nitric oxide have been tested and did not alter the change in synaptic fidelity seen with Panx1 blocking. Other potential retrograde signals are under investigation. We conclude that postsynaptic Panx1 are intimately linked to pre-synaptic glutamate release through an, as yet, unidentified mechanism.

Disclosures: J. Bialecki: None. **N.L. Weilinger:** None. **M.N. Hill:** None. **R.J. Thompson:** None.

Poster

038. Short Term Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 38.12/B83

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01MH098534

NIH Grant R56MH065328

Title: Differences in short-term plasticity cause a reduction in excitatory drive onto CA1 interneurons relative to pyramidal cells during temporally complex input patterns

Authors: *L. E. DOBRUNZ[1], Q. LI[1], A. F. BARTLEY[1], H. SUN[2];
[1]Dept. of Neurobio., Univ. of Alabama @ Birmingham, Birmingham, AL; [2]Dept. of Neurol., Univ. of Virginia, Charlottesville, VA

Abstract: Target-cell specific short-term plasticity enables Schaffer collateral (SC) synapses onto CA1 interneurons to have different dynamic properties than SC synapses onto CA1

pyramidal cells. We have previously shown that SC synapses onto CA1 interneurons have less paired-pulse facilitation than SC synapses onto pyramidal cells, and there is even a subset of interneurons that express paired-pulse depression. In contrast, there is little difference in the amount of steady-state high frequency depression between cell types. SC synapses onto pyramidal cells have robust facilitation in response to temporally complex stimulation patterns such as these synapses receive *in vivo*. However, little is known about how SC synapses onto interneurons respond to these patterns, or how short-term plasticity alters the relative amount of excitatory input onto interneurons compared to pyramidal cells. Here we compared SC synapses onto s. radiatum interneurons and CA1 pyramidal cells in acute hippocampal slices from juvenile rats in response to a physiologically derived Natural Stimulus Pattern (NSP). We find that SC synapses onto interneurons have less short-term facilitation and operate over a narrower dynamic range than synapses onto pyramidal cells, and a subset of interneurons has short-term depression. This causes a large reduction in the strength of the SC input onto interneurons relative to pyramidal cells, and of depression interneurons relative to facilitation interneurons, during high frequency periods of the NSP. This occurs to a similar extent at 25 °C and at 32 °C, but is even greater at a lower, more physiological relevant extracellular calcium level. We developed a simple mathematical model of short-term plasticity, which can fit this temporally complex data for all three cell groups at both temperatures and calcium concentrations. The model shows that target-cell specific differences in short-term plasticity can be accounted for by only a difference in the initial release probability between the three cell groups. Target-cell specific differences in initial release probability therefore enable SC synapses to have different temporal filtering characteristics, which may help to dynamically regulate the balance of inhibition and excitation in CA1.

Disclosures: **L.E. Dobrunz:** None. **Q. Li:** None. **A.F. Bartley:** None. **H. Sun:** None.

Poster

038. Short Term Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 38.13/B84

Topic: B.08. Synaptic Plasticity

Title: feedforward SHORT-TERM memory in Hippocampus: spine to network

Authors: ***S. YANG**[1], S. YANG[2], C.-M. TANG[3];
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Neurosci., University of California at San Francisco, CA; [3]Univ. of Maryland Sch. of Med., Maryland, MD

Abstract: The cellular mechanism underlying short term memory is a fundamental but poorly understood in neuroscience. Until now, synaptic modulation and reverberating activity within a feedback network have been the dominate models of short-term memory. Recently, a feedforward short-term memory (fSTM) model has been proposed which does not involve massive repetitive inputs. While mechanisms supporting fSTM was found at the level of the dendrite, they have not yet been demonstrated at the level of the spine and network. Here, using powerful techniques, including 3D digital holography with uncaging, voltage-sensitive dye imaging, two-photon calcium imaging, we show that a memory trace of past excitation can be held in the spines where this memory trace can be read-out at a later time in the form of calcium-delivering dendritic spikes. Such a sequence of events can mediate signal propagation throughout the whole hippocampal network. Our data thus provide a more thorough account of fSTM from spine to system in the hippocampus.

Disclosures: **S. Yang:** None. **S. Yang:** None. **C. Tang:** None.

Poster

038. Short Term Plasticity

Location: Hall A

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant MH046516

NIDA Grant DA007254-18

NIMH Grant F32MH100856-01

Title: Short-term plasticity as a homeostatic mechanism in the lateral amygdala

Authors: *A. E. FINK, T. J. MADARASZ, J. E. LEDOUX;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The lateral amygdala (LA) is an area that combines sensory information to produce both aversive and appetitive associative learning. Normally a quiet brain region, activity in the LA is thought to be dysregulated in disorders such as anxiety and PTSD. Short-term plasticity (STP) in the normally functioning LA might therefore be a crucial physiological mechanism for

maintaining amygdala homeostasis. STP (plasticity lasting from milliseconds to tens of seconds) modulates online processing in neuronal networks, and could also determine both the type of information that is passed through the circuit, as well as whether or not synaptic inputs can be incorporated into a memory engram. Nevertheless, little is known about short-term plasticity in the LA, whether it differs by input, and how it differs by frequency. These questions are important for understanding the LA microcircuit in health and disease, and we have conducted patch-clamp recordings in acute brain slices from adult male mice to identify properties of STP in the LA. We show that during trains of synaptic stimulation at theta (5 Hz), beta (30 Hz), or gamma (100 Hz) frequencies, synaptic inputs onto LA cells undergo a rapid, transient depression that varies both by frequency and by input. This depression is increasingly pronounced at higher frequencies of stimulation. Most importantly, our data suggest that these forms of STP arise from a combined modulation of excitatory input, inhibitory input, and biophysical properties of LA principal cells. We furthermore demonstrate that STP is robust to noradrenergic modulation. Specifically, we find that alpha1 and beta1-adrenergic agonists act primarily through changing excitatory-inhibitory balance, without interfering with frequency-dependent forms of STP.

Disclosures: **A.E. Fink:** None. **T.J. Madarasz:** None. **J.E. LeDoux:** None.

Poster

038. Short Term Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 38.15/B86

Topic: B.08. Synaptic Plasticity

Title: Paired-pulse modulation of axonal spikes at the hippocampal mossy fibers

Authors: *H. KAMIYA;
Hokkaido Univ. Grad. Sch. of Med., Sapporo, Hokkaido, Japan

Abstract: Use-dependent modification of synaptic strength is caused by changes in the probability transmitter release from the presynaptic terminals or in the responsiveness of the postsynaptic neurons. Axonal spike is the important upstream process of synaptic transmission which directly impacts the presynaptic release probability. However, its activity-dependent modulation has been less studied experimentally. Direct recordings from single axon enable testing possible use-dependent modification of axonal spikes. In acute hippocampal slices, the glass recording pipette was placed on the visually identified giant mossy fiber boutons located in the stratum lucidum of the CA3 region. Using a loose-patch clamp configuration from single presynaptic boutons, stimulation of the granule cell layer of the dentate gyrus elicits spikes

which occur in all or none fashion. This response was recorded also in the calcium free perfusing solution, confirming that they originated in the presynaptic component of the mossy fiber synapses. Unexpected from the digital nature of action potential, the peak amplitude of the second spikes in response to paired stimuli at ~50 ms interval was slightly but reproducibly smaller than the first spikes. This paired-pulse depression of the amplitude of axonal spikes may cause reduction of presynaptic calcium influx, and thereby reduce the transmitter release from the presynaptic terminals. Hippocampal mossy fiber boutons are reported to express highly abundant voltage-dependent sodium channels, although the density may not be sufficient to support fully digital signaling of axonal spikes.

Disclosures: H. Kamiya: None.

Poster

038. Short Term Plasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 38.16/B87

Topic: B.08. Synaptic Plasticity

Support: KAKENHI26430063

Title: CaMKIIβ is localized in dendritic spine in a drebrin-dependent and drebrin-independent manners

Authors: *H. YAMAZAKI[1], T. SHIRAO[2];
[2]Dept. of Neurobio. and Behavior, [1]Gunma Univ. Grad. Sch. of Med., Maebashi, Japan

Abstract: Dendritic spines are actin-rich small protrusions that contain postsynaptic components of excitatory synapse. Many actin-binding proteins have been identified as spine-resident protein, and they regulate actin-cytoskeleton through diverse processes. Drebrin is a major F-actin binding protein in neurons, and is localized in the center of dendritic spines. Drebrin regulates dendritic spine morphogenesis and spine targeting of synaptic proteins such as spikar, PSD-95 and NMDA receptors. Moreover, drebrin is involved in neurological diseases (eg., Alzheimer's disease and schizophrenia). Although increasing evidences show that drebrin plays pivotal roles in neurons, how drebrin interacts with other proteins in spines is much less known. In this study, we isolated CaMKIIβ as a drebrin-binding protein by yeast two-hybrid screen and investigated the interaction of drebrin-CaMKIIβ in dendritic spines. CaMKIIβ is localized in dendritic spines more than in dendritic shaft. However, drebrin knockdown (KD) caused diffuse localization of CaMKIIβ in dendrites, suggesting that drebrin anchors CaMKIIβ in dendritic

spines. To analyze drebrin-dependence of CaMKIIβ stability in dendritic spine, we performed fluorescence recovery after photobleaching (FRAP) experiments on individual dendritic spines. We calculated the stable fraction from the time-series of fluorescence intensity of GFP-CaMKIIβ before and after photobleaching. The stable fraction of GFP-CaMKIIβ in drebrin-KD neurons was greater than that of control neurons. In addition, NMDA receptor stimulation increased the stable fraction of CaMKIIβ in parallel with drebrin-dislocation from dendritic spines. These results suggest that drebin-loss increases the stable fraction of CaMKIIβ in dendritic spines. Therefore, we think that drebrin-independent stable pool became dominant in drebrin-KD neurons and synaptic activity regulates the accumulation of drebrin-independent CaMKIIβ in dendritic spines. Taken together, our study suggests that there are two stable pools of CaMKIIβ in dendritic spines, drebrin-dependent and drebrin-independent pools.

Disclosures: H. Yamazaki: None. **T. Shirao:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.01/B88

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institutes of Health Research MOP 93601

University Hospital Foundation

Alberta Innovates-Health Solutions

Title: Microglial amylin receptors: a novel target for the actions of beta amyloid (Aβ) protein

Authors: *J. H. JHAMANDAS, V. VUKOJEVIC, D. MACTAVISH, W. FU;
Univ. of Alberta, Edmonton, AB, Canada

Abstract: Alzheimer’s Disease (AD) pathology is characterized by amyloid deposits, neurofibrillary tangles, and activation of microglia. Our laboratory has shown that the toxic effects of amyloid beta (Aβ) protein on neurons are expressed, in part, through the amylin receptor, that is comprised of dimers of calcitonin receptor (CTR) and receptor activity modifying protein 3 (RAMP3). We have identified the presence of amylin receptors on human fetal microglia (HFMs) that are key immune cells in the CNS. In this study, we sought to identify a functional role for these microglial amylin receptors. Purified HFM cultures were first incubated with an *in vivo* microglial marker, DyLight 594 conjugated tomato lectin and loaded

with 5μM of the membrane-permeant green fluorescent dye, Fluo-8L-AM for measurements of intracellular calcium [Ca2+]i. Acute applications of human amylin (1μM) or Aβ1-42 (1μM) resulted in an increase in intracellular calcium levels that could be blocked by either the amylin receptor antagonist, AC253 (1μM) or pramlintide (1μM). RT-PCR measurements on HFMs exposed to either human amylin (1 μM) or Aβ1-42(1 μM) for 24 hours revealed an increase in the release of cytokines TNFα, IL-1β and IL-10, effects that could be blocked with AC253. Finally, application of either human amylin or Aβ1-42 resulted in an activation of the NLRP3 inflammasome complex (comprising of NLRP3, ASC and caspase-1), that was diminished by AC253 pretreatment of HFMs. This study provides first evidence for the presence of amylin receptors on microglia and identifies a functional role for these receptors in mediating human amylin or amyloid-evoked release of inflammatory mediators. Blockade of microglial amylin receptors with antagonists offers an attractive therapeutic target for intervention in AD on account of their anti-inflammatory effects identified in the present study.

Disclosures: **J.H. Jhamandas:** None. **V. Vukojevic:** None. **D. MacTavish:** None. **W. Fu:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.02/B89

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: T-cell mediated inflammation is involved in pathogenesis in Alzheimer’s disease mouse model

Authors: ***J. BLOEMER**, M. AHUJA, E. ABDEL-REHMAN, D. BHATTACHARYA, S. BHATTACHARYA, A. ALHOWAIL, F. ALJADANI, D. KATZ, R. AMIN, V. SUPPIRAMANIAM, M. DHANASEKARAN;
Drug Discovery and Develop., Auburn Univ., Auburn, AL

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative cognitive disorder, which is an increasing problem in the aging population. Although amyloid-beta theory is considered a cornerstone of AD pathogenesis, it does not explain all the aspects of AD and thus the exact cause of AD remains elusive. Inflammation is considered to be one of the pathological factors associated with AD, however there is a lack of experimental evidence on the mechanisms involved. Therefore, this study was carried out to better understand and establish the pathophysiological involvement of chronic inflammation in AD. Ten-month-old mutated APΔE9

mice, along with wild type control C57BL/6 mice, were assessed for behavioral, biochemical, and synaptic deficits. Amyloid-beta (Aβ) levels and beta sectretase (BACE) activity was assessed biochemically. Synaptic plasticity was assessed by extracellular field recordings, and changes in synaptic excitatory receptors were evaluated by western analysis. We also analyzed the pro-inflammatory cytokines, chemokines, and T-cells in different regions of the brain through various immunological and biochemical techniques. Substantial increase in Aβ levels together with increased BACE activity was observed in 10 month old transgenic mice as compared to age matched control. Marked elevations in pro-inflammatory cytokines in cortical and whole brain lysates were observed. Robust T-cell infiltration and activation in the transgenic mice brains were observed as evidenced by higher frequency of CD4+ IL-17a secreting T-cells. Behavioral deficits in learning and memory tasks, specifically Y-maze and novel object recognition, were also exhibited along with impaired long-term potentiation (LTP) in APΔE9 mice compared to WT mice. Taken together, the current study establishes the presence of T-cell mediated neuroinflammation associated with increased Aβ deposition in the APΔE9 mouse model, which may be the cause of cognitive decline and behavioral deficits in these mice.

Disclosures: **J. Bloemer:** None. **M. Ahuja:** None. **E. Abdel-Rehman:** None. **D. Bhattacharya:** None. **S. Bhattacharya:** None. **A. Alhowail:** None. **F. Aljadani:** None. **D. Katz:** None. **R. Amin:** None. **V. Suppiramaniam:** None. **M. Dhanasekaran:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.03/B90

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH GM060665

NIH MD007599

PSC-CUNY 67851-00 45

The Graduate Center, CUNY

Title: Inflammation alters APP glycosylation relevant to Alzheimer’s disease

Authors: ***T. JEAN-LOUIS**[1,2], P. ROCKWELL[1], M. FIGUEIREDO-PEREIRA[1];
[1]Biol. Sci., Hunter Col., New York, NY; [2]The Grad. Center, CUNY, New York, NY

Abstract: Senile plaques are important pathological hallmarks of Alzheimer disease (AD). The main component of senile plaques is Aβ, a polypeptide generated via the sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretases in the amyloidogenic pathway. Alternatively, APP processing by α-secretase in the non-amyloidogenic pathway, which prevents Aβ formation. The upstream events that regulate APP processing by both the amyloidogenic and non-amyloidogenic pathways are poorly defined. We propose that inflammation is a mechanism that shifts the balance towards APP processing by the amyloidogenic pathway. Inflammation plays a major role in AD. Investigating how specific factors of inflammation mediate the neurodegenerative processes in AD is crucial. Our studies focus on prostaglandin products of cyclooxygenases, which are key enzymes in inflammation and highly relevant to AD. In particular, we are investigating how the neurotoxic prostaglandin J2 (PGJ2) alters the processing, trafficking, and post-translational modifications of APP. Here we report that PGJ2 affects the post-translational glycosylation of APP in rat cerebral cortical neuronal cultures. Investigating APP glycosylation in neurons is important because (1) glycosylation deficits in APP have been detected in brains of AD patients, and (2) changes in APP glycosylation may trigger a shift from the non-amyloidogenic to the amyloidogenic pathway. Neuronal APP occurs as a mature form, exhibiting both N- and O-glycosylation, as well as an immature form, displaying only N-glycosylation. APP processing seems to be affected by glycosylation, as secretases preferentially cleave APP once it is O-glycosylated. In our studies, we established that PGJ2 decreases the levels of APP O-glycosylation in a time and concentration-dependent manner. The levels of N-glycosylated APP were not affected by the PGJ2 treatment. The finding that N-glycosylation is not changed and O-glycosylation is significantly decreased may indicate that immature APP is sequestered in the ER and unable to translocate to the Golgi for O-glycosylation. Furthermore, this PGJ2-induced blockade of APP translocation to the Golgi is likely to induce the ER stress (UPR) response. In conclusion, alterations in APP glycosylation induced by the endogenous product of inflammation PGJ2 is likely to have an impact on the intracellular sorting, processing and export of APP that are affected in AD.

Disclosures: **T. Jean-Louis:** None. **P. Rockwell:** None. **M. Figueiredo-Pereira:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.04/B91

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FONDECYT Grant 1131025

Title: Gender related cognitive differences in response to chronic inflammation and passive immunotherapy in an Alzheimer's disease mouse model

Authors: *L. EUGENIN-VON BERNHARDI[1,2], A. PEÑAILILLO[2], N. SALGADO[2], R. VON BERNHARDI[1,2];
[1]Neurología, [2]Lab. de Neurociencias, Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract: Alzheimer's disease (AD) is the most common dementia among the elderly, with increasing prevalence over the last years. Despite all the research directed to understand this pathology and generate effective therapies, its molecular mechanisms have not been fully understood and no therapeutic target has been proven useful. Presently, the most accepted hypothesis for AD, the "amyloid cascade hypothesis", coexists with a new proposal, progressively empowered, the "dysfunctional glial cell hypothesis". Our laboratory has worked on this second proposal over the last eight years, showing that microglia and astrocytes have a key role in the pathogenesis of AD. Age-related inflammatory state - via redox imbalance and innate immunity activation- ultimately results in a cytotoxic activation of glia and neurodegeneration. Chronic inflammation in APP/PS1 mice is associated with impaired Aβ-plaques clearance by microglia, a central feature of Alzheimer's disease histopathology. Here, immunotherapy and inflammatory treatment consisted in consecutive biweekly i.p. injections of APP/PS1 (APPswe, PSEN1dE9) mice with anti-Aβ antibodies and/or LPS for 3 months. After treatment, mice were trained in the Morrison's water maze neurobehavioral tasks. We observed that APP/PS1 mice exposed to chronic inflammatory states had worst cognitive performance in reference and episodic memory tasks (traduced as an increased latency duration) in comparison with APP/PS1 mice receiving saline injections and wild type mice under similar conditions. In contrast, APP/PS1 mice receiving anti-Aβ passive immunotherapy performed better than those without anti-Aβ antibody treatment. Furthermore, we observed a gender difference among all the subgroup treatments. Female mice appeared to learn faster to recognize the platform than males, but did not improve their latency times as males did. These differences were enhanced in APP/PS1 female mice that received chronic inflammatory treatments. We have also shown that immunotherapy affects microglial cell activation, improving their phagocytic activity on Aβ. Our work is coherent with cognitive changes observed in other mice models although human patients have failed to show improvement in response to immunotherapy. By means of this *in vivo* study on cognitive function, and our results on the effect of immunotherapy on the activation pattern of glia, we propose to correlate neurobehavior with pathophysiological mechanisms related with microglial cell activation.

Disclosures: L. Eugenin-Von Bernhardi: None. **A. Peñailillo:** None. **N. Salgado:** None. **R. von Bernhardi:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.05/B92

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FONDECYT Grant 1131025

Title: Effect of inflammation and anti-Aβ immunotherapy on the pattern of microglia activation and their association with β amyloid plaques in an Alzheimer's disease animal model

Authors: *R. VON BERNHARDI, N. SALGADO, G. RAMÍREZ, P. NAVARRO;
Pontificia U Catolica De Chile, Fac Med., Santiago, Chile

Abstract: β-amyloid (Aβ) immunotherapy has beneficial effects on Alzheimer disease (AD) mice models. However, immunotherapy trials in AD patients have mostly failed. We propose that AD depends on age-related dysregulation of microglia resulting in a cytotoxic environment and impairment of scavenger functions. We assessed the effect of passive immunotherapy on neuroinflammation, changes on microglia activation and the association with Aβ plaques of microglia. Immunotherapy and inflammatory treatment consisted in biweekly i.p. injections of APP/PS1 (APPswe, PSEN1dE9) mice with anti-Aβ antibodies and/or LPS for 3 m. Microglia activation was assessed by ELISA of inflammatory cytokines (IL1β, TNFα and TGFβ) in brain tissue, and the expression pattern of various activation markers (CD68, FcR, Iba-1, MHC-I and MHC-II) in hippocampal microglia, by immunohistochemistry and stereology. Aggregation of microglia in proximity to Aβ plaques labeled with thioflavin was also assessed. Stereological analysis showed that the density of cells with the constitutive marker Iba-1(+) was similar for WT and APP/PS1 mice, indicating that the number of microglia was unaffected. In contrast, CD68(+), MHC-I(+) and MHC-II(+) cells showed a 100% increase in APP/PS1 mice. In un-stimulated APP/PS1 mice predominated CD68(+) cells, whereas MHC-II(+) was nearly absent. Treatment with LPS and immunotherapy did not affect CD68 expression. However, both Inflammation and immunotherapy resulted in a 50% increase of MHC-I(+) cells, whereas MHC-II(+) cells showed a 3-fold decrease for the immunotherapy plus LPS treatment. APP/PS1 mice that received anti-Aβ immunotherapy showed a 30% reduction on total Aβ deposition in the hippocampus. However, there was only a mild effect on microglia associated plaques, which varied depending on their activation pattern. Iba-1(+) and FcR(+) cells increased after immunotherapy, but that population was reduced in response to LPS, whereas MHC-I(+) cells increased after immunotherapy plus LPS and MHC-II(+) cells were increased under inflammatory conditions. Finally, the association of CD68(+) microglia with plaques was reduced by both immunotherapy and LPS. It was especially interesting the increase of MHC-I(+) and the decrease of MHC-II(+) cells induced by both immunotherapy and systemic inflammation. However, MHC-II(+) cells showed a 3-to-10 fold higher association with plaques

than MHC-I(+) cells, which was increased by the inflammatory stimulation. Our results suggest that passive immunotherapy can immunomodulate microglia, leading to changes in their activation pattern and decrease in the number and size of amyloid plaques.

Disclosures: R. von Bernhardi: None. **N. Salgado:** None. **G. Ramírez:** None. **P. Navarro:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.06/B93

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG042082

NIH Grant AG030399

Title: The role of the TRIF-dependent pathway in β-amyloidosis and neuroinflammation in a mouse model of Alzheimer’s disease

Authors: J.-E. LIM[1], J. YANG[1], J. KOU[1], R. LALONDE[2], *K.-I. FUKUCHI[1];
[1]Cancer Biololgy and Pharmacol., Univ. of IL Col. of Med. At Peoria, Peoria, IL; [2]Dept. of Psychology, Univ. of Rouen, Rouen, France

Abstract: Toll-like receptors (TLRs) are a class of pattern-recognition receptors in the innate immune system. One of the important roles of TLRs is to activate phagocytes/microglia in response to pathogens and damaged host cells, and to clear pathogens, damaged tissues, and accumulated wastes. Some TLRs, including TLR2, TLR4, and TLR6, have been shown to be essential components of the receptor complexes for microglial activation by Aβ. All TLRs use MyD88 as an adaptor except TLR3. The ligation of TLR2 and TLR4 culminates in activation of transcription factors, NF-κB and AP1, through the MyD88-dpendent pathway that is essential for expression of cytokines, chemokines and co-stimulatory molecules, such as TNF-α, IL-1β, IL-6, IL-8, IL-12 and MIP1α. TLR3 and TLR4 ligation can signal via the TRIF-dependent pathway, leading to the activation of interferon regulatory factor 3 (IRF3) that induces expression of type I interferon (IFN) genes such as IFNβ and IFN-inducible genes. It remains to be determined which TLR signaling pathways and effectors are involved in modulation of Aβ deposition and clearance in the brain. In this study, we tested whether activation of the TRIF-dependent pathway is involved in Aβ accumulation and neuroinflammation because TLR4 is an essential component

of the cell surface receptor complex for fibrillar Aβ recognition by microglia. Polyadenlyic-polyuridylic acid, poly(A:U), is a synthetic double stranded RNA molecule that signals only through the TRIF-dependent pathway. We intraperitoneally injected poly(A:U) into an AD mouse model. Currently, we are investigating Aβ load and inflammatory responses in experimental animals to determine the role of the TRIF pathway in the AD pathogenesis.

Disclosures: J. Lim: None. **J. Yang:** None. **J. Kou:** None. **R. Lalonde:** None. **K. Fukuchi:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.07/B94

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 7thFP EUPRIM-Net Contract 262443

Title: Acceleration of amyloidosis by inflammation in the amyloid-beta marmoset monkey model of Alzheimer’s disease

Authors: *I. H. PHILIPPENS;
Biomed. Primate Res. Ctr. (BPRC), Rijswijk, Netherlands

Abstract: Background: A pro-inflammatory state is increasingly associated and mentioned for its therapeutic potential to target amyloidopathy that characterizes Alzheimer’s disease (AD). The common marmoset monkey has potential as an AD model due to its natural amyloidosis. Moreover, the marmoset shows a human-like immune system and aging phenotype, which is partly due to exposure to environmental pathogens causing transient or chronic latent infections. Aim was to investigate the effect of inflammation on amyloidopathy in the marmoset AD model. Methods: Four middle aged (5-8y) and two aged (13-14y) common marmoset monkeys (Callithrix jacchus) of both sexes were intracranial injected with amyloid-beta (Aβ) fibrils at three cortical locations into the right hemisphere (frontal, parietal, and sensorimotor cortices) and both hemispheres were injected with PBS (n=3) or LPS (n=3). The effect of inflammation on amyloidopathy was also investigated in an animal that died due to a systemic inflammatory condition, marmoset-wasting syndrome (MWS), which is associated with chronic colitis. The pro-inflammatory effect of LPS and Aβ was also tested in an ex vitro blood analysis (flow cytometry) for immune cell biomarkers (CD45RA and CD95). Campbell-Switzer silver staining and IHC analyses (Aβ, Aβ42, Aβ43, Iba1, and GFAP antibodies) were used on mirror sections to

assess amyloidopathy and immune reaction. Results: The MWS and two LPS+Aβ monkeys developed plaques within 5 months and all LPS+Aβ animals had an early-AD immune blood cell expression profile as seen in human AD patients. Conclusion: A pro-inflammatory condition accelerates amyloidopathy in the marmoset, which indicates the possible importance of immune modulation to decrease the susceptibility for AD. This study was supported by the EU transnational access to the research infrastructure PRIMOCID-205 of EUPRIM-Net under the EU contract 262443 of the 7th Framework Program

Disclosures: I.H. Philippens: None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.08/B95

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer Society of Canada

Canadian Institutes of Health Research

Title: Changes in amyloid deposition and neuroinflammation with age and its relationship with learning deficits in a mouse model of Alzheimer’s disease

Authors: *S. ZHU[1], J.-F. WANG[2], X.-M. LI[3];
[1]Dept. of Pharmacol. and Therapeut., [2]Pharmacol. and Therapeut., Univ. of Manitoba, Winnipeg, MB, Canada; [3]Psychiatry, Univ. of Alberta, Edmonton, AB, Canada

Abstract: The pathology of Alzheimer’s disease (AD) includes amyloid plaques and neurofibrillary tangles as well as chronic neuroinflammation characterized by frank microglial/astroglial activation and subsequent upregulation of proinflammatory cytokines. Both amyloid deposition and neuroinflammation appear in the early course of AD and become notably conspicuous as disease progresses. However, the progression of neuroinflammation and its relationship with amyloid deposition and behavioural changes have not been characterized as many underlying mechanisms rarely occur in isolation. The present study will thoroughly characterize the behaviour of the APP/PS1 mouse model of AD, using a comprehensive test battery designed to assess a variety of behaviours including: working memory, reference memory, long-term memory, anxiety, and motor ability. Using a cross-sectional design, these behaviours will be assessed in mice aged 2-3 months, 6-7 months, 9-10 months, 12-13 months,

and 15-16 months. Brain pathology measures for amyloid deposition and neuroinflammation are done post-mortem. APP/PS1 mice exhibited significant learning deficits from the age of 6 month, which were aggravated at the later stages of life. However, the degree of memory impairment plateaus after 12 months. Histological analyses showed that an early appearance of amyloid plaques at 3 months of age with a linear progressive increase up to 22 months. This pronounced amyloid deposition was accompanied by a steady increase of the glial fibrillary acidic protein (GFAP) positive astrocytes and CD11b positive microglia up to the age of 9-12 months. Interestingly the expression levels of GFAP rose steeply from the age of 5 months to the age of 9 months and then stabilized at the age of 12 months which coincided with the observed pattern of learning deficits in the APP/PS1 mice. This work aims to provide advances in the knowledge of the neuroinflammatory changes and its relationship with amyloid deposition and behavioural changes that occur in AD patients.

Disclosures: **S. Zhu:** None. **J. Wang:** None. **X. Li:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.09/B96

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Oleamide identified in a fermented dairy product activates microglial phagocytosis via peroxisome proliferator-activated receptor γ and suppresses inflammatory response via cannabinoid receptor type 2

Authors: *Y. ANO[1], T. KUTSUKAKE[1], M. KITA[1], K. UCHIDA[2], H. NAKAYAMA[2]; [1]Central Lab. for Key Technologies, Kirin Company, Limited, Yokohama-Shi, Japan; [2]Dept. of Vet. Pathology, the Univ. of Tokyo, Tokyo, Japan

Abstract: Despite the ever-increasing number of patients with dementia worldwide, fundamental therapeutic approaches to this condition have not been established. Epidemiological studies suggest that intake of fermented dairy products prevents cognitive decline in the elderly. We, previously, reported that in a mouse model of Alzheimer's disease (5xFAD) intakes of camembert cheese reduce the accumulation of amyloid β (Aβ) and hippocampal inflammation. In the present study, we searched the responsible components in dairy products with primary microglial phagocytosis and anti-inflammatory assay systems. As a result of screening, oleamide was identified as a novel dual-active component in camembert cheese that enhanced microglial Aβ phagocytosis and suppressed TNF-α and MIP-1α production and MHC class II and CD86

expression towards lipopolysaccharide (LPS) stimulation. Orally administered oleamide (10 and 50 mg/kg) also enhanced microglial Aβ phagocytosis in hippocampus, and reduced TNF-α production after LPS i.c.v. injections. Oleamide is also known as an endogenous substance that binds to cannabinoid (CB) receptors as an agonist and known as a sleep-inducing property. In this study, we elucidated novel oleamide functions modulating microglial activities. As a result of GC/MS analysis, oleamide was identified in various fermented products such as gorgonzola cheese, cheddar cheese, and aging beef, as well as camembert cheese. Oleamide is the amide of oleic acid abundant in milk, and is synthesized from oleic acid and ammonia by enzymatic amidation during fermentation. We also elucidated that enhancement of microglial Aβ phagocytosis by oleamide was diminished by the treatment of an antagonist of peroxisome proliferator-activated receptor γ (PPAR-γ) and that suppression of microglial TNF-α production by oleamide was partly diminished by the treatment of an antagonist of CB receptor type 2 (CB2) *in vitro*. Therefore, the activation of oleamide, enhanceing microglial phagocytosis and anti-inflammatory activity, may be mediated via the PPAR-γ and CB2 receptor, respectively. The present study demonstrated that oleamide is an active component of fermented dairy products that reduces the Aβ accumulation via enhanced microglial phagocytosis and suppresses microglial inflammation after the Aβ deposition. Oleamide contained in fermented foods such as cheese is easy to intake as a daily meal, and would be an effective prevention against dementia.

Disclosures: Y. Ano: A. Employment/Salary (full or part-time):; Kirin Company, Limited. **T. Kutsukake:** A. Employment/Salary (full or part-time):; Kirin Company, Limited. **M. Kita:** A. Employment/Salary (full or part-time):; Kirin Company, Limited. **K. Uchida:** A. Employment/Salary (full or part-time):; the University of Tokyo. **H. Nakayama:** A. Employment/Salary (full or part-time):; the University of Tokyo.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RGC (GRF – 660813)

NIH grant (NS071022)

National Key Basic Research Program of China (2013CB530900)

Title: Glial cells mediate cell cycle-related neuronal death induced by inflammatory challenge: evidence from a pure neuronal culture model

Authors: *Y. ZHANG, C. HUI, K. HERRUP;
Hong Kong Univ. of Sci. and Technol., Hong Kong, Hong Kong

Abstract: Chronic inflammation characterized by activated microglia and reactive astrocytes plays an important role in the pathogenesis of neurodegenerative diseases, especially in Alzheimer's. Previous studies in our lab have demonstrated that beta-amyloid activated microglia or THP-1 monocytes secrete diverse bioactive molecules, most probably cytokines to induce cell cycle-related death in cultured mouse cortical neurons. In order to investigate the role of other glial cells, especially astrocytes in establishing this effect, we developed a modified method to remove the glial cells in primary culture from E16.5 mouse cortex. We use a brief treatment of the thymidine analog, 5-fluorodeoxyuridine (FdU), to kill the proliferating cells in culture. Two weeks later, cell cycle and glial markers confirmed the loss of ~99% of all microglia, astrocytes and NG2 glia. More importantly, no morphological defects were observed at DIV15-21; both pre- and post-synaptic markers were retained in the purified neuron culture. FdU-treated culture maintained responsiveness to excitotoxicity induced by L-glutamate application, and presumably had normal synaptic function. By applying individual cytokines or conditioned medium from THP-1 cells stimulated by beta-amyloid, we showed that the absence of glial cells dramatically attenuated cell cycle related death in cultured neurons. This observation could be explained by altered immunobiology in FdU culture. Compared with mixed culture, both protein levels and activity of NFκB p65 were decreased, suggesting a reduced NFκB activity. Besides, the gene expression of several cytokine receptors in FdU culture was significantly altered. Thus, we have developed a modified FdU protocol to obtain purified neuronal cultures. With this model, we demonstrate that the presence of glial cells, most probably astrocytes, are required for cell cycle-related neurodegeneration induced by certain inflammatory challenges, including individual cytokines or a mixture of different cytokines and chemokines. We propose to further investigate how astrocytes mediate cell cycle-related death this process. Whether biochemical or physical neuron-glial interaction was required will be determined in the future.

Disclosures: **Y. Zhang:** None. **C. Hui:** None. **K. Herrup:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA Merit Review I01BX002477

NIH Grant R01NS073670

Title: Distribution of glia maturation factor and mitochondrial uncoupling protein-2 and -4 in neuronal degeneration

Authors: *A. ZAHEER[1,3], R. THANGAVEL[2], D. KEMPURAJ[3], S. ZAHEER[1];
[1]Neurol., [2]Univ. of Iowa, Iowa City, IA; [3]VAHCS, Iowa City, IA

Abstract: Alzheimer's disease (AD) is characterized by the presence of neuropathological lesions containing amyloid plaques (APs) and neurofibrillary tangles (NFTs). AD is associated with mitochondrial dysfunctions, neuroinflammation and neurodegeneration in the brain. Our previous immunohistochemical staining in temporal cortex showed enhanced expression of proinflammatory protein glia maturation factor (GMF) in glial cells at the vicinity of APs and NFTs in AD brains. Parahippocampal gyrus consisting entorhinal and perirhinal subdivisions of temporal cortex is the first brain region affected during AD pathogenesis. In the present study, we have analyzed the expression of GMF and mitochondrial proteins (UCP) 2 and 4 in the parahippocampal gyrus of AD and non-AD brains by immunostaining techniques. APs were detected by thioflavin-S fluorescence staining or 6E10 antibody. Results showed down-regulation of UCP2 as well as UCP4, and upregulation of GMF expression in the parahippocampal gyrus of AD brains when compared to non-AD brains. GMF expression is associated with up-regulation of inducible nitric oxide synthase (iNOS), the enzyme that induces the production of nitric oxide, as well as nuclear factor kB (NF-kB p65) expression. In conclusion, increased expression of GMF may down-regulate UCP2 and UCP4 in AD. Increased GMF and its co-localization with iNOS and NF-kB suggest that GMF may play proinflammatory role in the pathogenesis of AD.

Disclosures: **A. Zaheer:** None. **R. Thangavel:** None. **D. Kempuraj:** None. **S. Zaheer:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.12/B99

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Investigating the role of glia in the amyloid beta-associated pathogenesis of Alzheimer's disease using *Drosophila* as a model system

Authors: *A. RAY, E. J. MUENZEL, S. D. SPEESE, M. A. LOGAN;
Jungers Center, Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Glial cells are tightly coupled to the onset and progression of many neurodegenerative diseases. In the context of Alzheimer's disease (AD), glia play a protective role by engulfing and digesting neurotoxic amyloid-β (Aβ) peptides and eventually clearing degenerating neurons that succumb to disease, but the precise molecules that regulate glial responses to Aβ remain unclear. Glial cells likely express a repertoire of cell surface receptors involved in recognition, internalization, and clearance of Aβ. Although glial responses to Aβ have been extensively examined *in vitro*, the complexity of the CNS has hindered our understanding of how glia might recognize and destroy Aβ *in vivo*. Using *Drosophila melanogaster* as a well-established system to investigate amyloid proteinopathies, we find that transgenic expression of the neurotoxic human $A\beta42^{arc}$ fragment in neurons results in age-dependent Aβ accumulation, neurodegeneration and locomotor defects, as well as reduced lifespan. Interestingly, electron microscopy reveals that Aβ aggregates are present in *Drosophila* glia following neuronal expression of Aβ, suggesting that fly glia internalize secreted Aβ peptides (Iijima et al, 2008), but the molecules involved in glial clearance of Aβ in flies are unknown. Draper/MEGF10 is a highly conserved receptor required for phagocytosis of apoptotic cells and degenerating neuronal projections (MacDonald et al, 2006; Wu et al, 2009; Chung et al, 2013). Based on an *in vitro* study, the mammalian homolog MEGF10 may also be involved in Aβ internalization (Singh et al, 2010). Thus, we hypothesize that Draper is required for glial engulfment of Aβ in the fly CNS. Interestingly, by performing anti-Draper immunostaining we find that glial Draper is significantly increased ($p<0.05$) in the central brain following neuronal expression of $A\beta42^{arc}$. Also, since the transcription factor STAT is a known regulator of *draper* expression (Doherty et al, 2014), we asked if STAT activity was upregulated in Aβ-expressing flies. Indeed, our preliminary data using an *in vivo* reporter for STAT function suggest that Aβ expression triggers STAT transcriptional activity, although the cell autonomy of this response remains to be determined. Currently, we are using genetic strategies to perturb glial phagocytic function *in vivo* (e.g. *draper* null mutants, RNAi knockdown of additional scavenger receptors, etc) to determine how Aβ aggregation, neurodegeneration and longevity are affected. This work will provide important molecular insight into how glia recognize and destroy Aβ and also allow us to explore the protective role that glia play in delaying Aβ-mediated neurodegeneration *in vivo*.

Disclosures: A. Ray: None. **E.J. Muenzel:** None. **S.D. Speese:** None. **M.A. Logan:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01NS079637-01

Title: Determining the neuroinflammatory phenotype in a model of amyloid deposition and vascular cognitive impairment after anti-Aβ immunotherapy

Authors: *C. N. CAVERLY, E. M. WEEKMAN, T. J. KOPPER, T. L. SUDDUTH, D. M. WILCOCK;
Physiol., Univ. of Kentucky, Lexington, KY

Abstract: Alzheimer's disease (AD) and vascular contributions to cognitive impairment and dementia (VCID) are the two most common forms of dementia and it is estimated that 40% of AD patients also have VCID. Several studies have described the heterogeneity of neuroinflammatory phenotypes in AD and the effects of these states on the pathologies of the disease. Our laboratory has recently shown an M1 phenotype dominates when VCID is co-morbid with amyloid deposition in a mouse model. Due to the disappointing outcomes of anti-Aβ immunotherapy clinical trials, and the significant vascular adverse events in these trials we hypothesized that anti-Aβ immunotherapy could result in an adverse neuroinflammatory response when AD and VCID are co-morbid leading to adverse events and reduced efficacy. To model AD-VCID co-morbidity we use the APP/PS1 mouse model of amyloid deposition and induced hyperhomocysteinemia (HHcy) via diet, which models a form of VCID. For this study, we placed 9 month old wildtype or APP/PS1 mice on a control diet or the HHcy diet. After 3 months on diet, when cerebrovascular pathology is induced by the HHcy, the mice received weekly intraperitoneal injections of a control antibody (IgG2a) or N-terminal anti-Aβ antibody (3D6). The neuroinflammatory phenotype was assessed by qPCR for gene markers specific for inflammatory phenotypes. Protein levels via ELISA and microglial activation via CD11b immunohistochemistry are currently in progress. We found that the APP/PS1 mice on control diet with 3D6 treatment were polarized towards an M1/M2b phenotype. Interestingly, of the genes tested, the APP/PS1 mice on the HHcy diet with 3D6 treatment showed significant exacerbation of many neuroinflammatory markers and we are continuing to assess these more carefully. Overall, we found that anti-Aβ immunotherapy in a model of amyloid deposition and VCID results in a different neuroinflammatory response than in a model of amyloid deposition alone.

Disclosures: C.N. Caverly: None. **E.M. Weekman:** None. **T.J. Kopper:** None. **T.L. Sudduth:** None. **D.M. Wilcock:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.14/B101

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association Grant NIRG-12-242598

Title: Molecular interaction between naturally secreted Amyloid β oligomers, GLT-1 downregulation and NF-kB activation in astrocytes

Authors: *J. M. ZUMKEHR, M. KITAZAWA;
Natural Sci., UC Merced, Merced, CA

Abstract: The excitatory amino acid transporter 2 or its mouse homologue glutamate transporter 1 (GLT-1, herein collectively referred to as GLT-1) is a major transporter responsible for regulating glutamate levels in the brain. We and others have suggested that its impairment can exacerbate pathology in many neurodegenerative diseases including Alzheimer's disease (AD). We recently reported that global GLT-1 levels significantly decrease when exposed to naturally secreted Amyloid β (Aβ) species in the conditioned media (CM) of 7PA2 Chinese hamster ovary (CHO) cells that express the human APP751 containing the V717F AD mutation in APP751 (7PA2-CM) *in vitro*. To further understand the underlying molecular mechanism, we examined the altered activation of several transcription factors responsible for Aβ -induced GLT-1 downregulation in the murine primary astrocyte and neuron co-culture system. Interestingly, we found that NF-κB p65/RelA nuclear translocation significantly decreased after forty eight hours of 7PA2-CM compared to CHO-CM (control). In addition, EMSA revealed less NF-κB activity in the nuclear fraction of astrocytes when exposed to 7PA2-CM compared to CHO-CM. These results suggest that NF-κB upregulates GLT-1 transcription under normal conditions while exposure to 7PA2-CM containing Aβ species counteracts this process. Our findings show a relationship between Aβ and GLT-1 downregulation mediated by NF-kB which may be a potential pathway target to prevent glutamate dyshomeostasis and thus prevent further exacerbation of Alzheimer's disease pathology.

Disclosures: J.M. Zumkehr: None. **M. Kitazawa:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.15/B102

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Training Grant (T32) AG00538

Title: C5a enhances the injury to primary neurons elicited by fibrillar amyloid beta

Authors: *M. X. HERNANDEZ, P. NAMIRANIAN, E. NGUYEN, A. J. TENNER; Pathology and Lab. Med., UC-Irvine, Irvine, CA

Abstract: Previous work demonstrated the C5aR1/CD88 antagonist, PMX205, decreased amyloid pathology and suppressed cognitive loss in two Alzheimer Disease (AD) mouse models. However, the molecular mechanism of this protection has not been definitively demonstrated. C5aR1 is expressed on microglia, and has also been reported to be expressed on endothethial cells and neurons in some environments. Others have demonstrated that C5a leads to injury to primary neurons using the trypan blue exclusion assay. Here, exogenous C5a is shown to lead to a dose-dependent loss of MAP-2 staining in primary murine neuron cultures within 24 hours of treatment, indicative of injury to neurons. This injury is prevented by the C5aR1 antagonist PMX53, a close analog of PMX205. To determine the influence of C5a in brain with accumulating fibrillar amyloid beta plaques (fAβ), we tested if C5a can enhance the injury to neurons initiated by fAβ *in vitro*. Primary neurons from C57B6 at E14.5 were cultured on glass coverslips for 7 days and treated with C5a with or without fAβ. PMX53 was added 30 minutes prior to addition of C5a to block C5aR1. After twenty four hours, cells were fixed and MAP-2 staining was used as a marker of injury to the primary neurons. Data shows low levels (1nM) of C5a can enhance the damage to neurons treated with 5uM fAβ. Blocking C5aR1 with the receptor antagonist PMX53 (100nM) blocked the loss of MAP2 in these primary neurons treated with fAβ and C5a. The data further adds to the growing literature on the contribution of C5a in neuroinflammation and the beneficial effects of C5aR1 antagonists as a therapeutic candidate for neurodegenerative diseases, particularly Alzheimer’s.

Disclosures: M.X. Hernandez: None. **P. Namiranian:** None. **E. Nguyen:** None. **A.J. Tenner:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.16/B103

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS079637 (DMW)

Title: Elevated homocysteine results in activation of microglia and pro-inflammatory cytokine production both *in vitro* and *in vivo*

Authors: F. GONZALEZ OREGON[1], T. L. SUDDUTH[1], E. M. WEEKMAN[1], *D. M. WILCOCK[2];
[1]Sanders-Brown Ctr. on Aging, Dept Physiol., [2]Univ. of Kentucky, Lexington, KY

Abstract: Homocysteine is a non-protein forming amino acid that is generated as a result of the methionine metabolism. It is a by-product that is normally maintained at low concentrations in the body through enzymatic clearance and recycling. Accumulation of homocysteine in the plasma, termed hyperhomocysteinemia (HHcy) is a risk factor for a vast array of conditions including cardiovascular disease, cerebrovascular disease and stroke, Alzheimer's disease (AD), and diabetes. Induction of HHcy in wildtype mice models vascular cognitive impairment and dementia (VCID). The exact mechanism by which HHcy increases risk for these conditions is unknown but a common pathology of all of these conditions is inflammation. We hypothesized that homocysteine activates microglia resulting in pro-inflammatory responses in the brain and therefore increasing susceptibility to diseases including AD and VCID. We treated BV2 microglial cells with increasing concentrations of homocysteine and examined their responses 24 hours after treatment. We found that the most robust changes were increased pro-inflammatory cytokine expression, specifically IL-1β, TNFα and IL6. M2-type markers of inflammation including IL1Ra and YM1 were also increased to a lesser extent. In wildtype mice, when HHcy is induced over several months we find that there is a significant inflammatory response characterized by increased expression of IL-1β, TNFα and IL6, as well as activation of the microglia as assessed by cell surface markers CD45 and CD11b. We have found, in a time course of pathology, that the inflammatory changes in the mouse model precede any pathological changes in the cerebrovasculature, perhaps suggesting a causal role of the neuroinflammatory changes in the onset and progression of VCID. Future studies include testing compounds for inhibition of homocysteine effects on BV2 *in vitro* and then taking these into the mouse model to assess their efficacy in ameliorating VCID pathologies.

Disclosures: F. Gonzalez Oregon: None. **T.L. Sudduth:** None. **E.M. Weekman:** None. **D.M. Wilcock:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.17/B104

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer Nederland Grant WE.15-2014-02

Title: Identifying stimulators of TYROBP-associated M2 microglial phagocytosis of amyloid beta *in vitro*

Authors: *J. DE VRY[1,2], P. VANDORMAEL[2,3], T. VANMIERLO[3], J. HENDRIKS[3], B. BRÔNE[2], J. PRICKAERTS[1];
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Abstract: Accumulation of neurotoxic extracellular amyloid beta (Aβ) in the brain is a major pathological hallmark of Alzheimer's disease (AD). Microglia are key players involved in Aβ phagocytosis and clearance, and alternatively activated microglia (M2) do this without provoking an inflammatory response. M2-associated TYRO protein tyrosine kinase-binding protein (TYROBP) regulation of phagocytosis was found to be hampered in AD, suggesting that reduced M2 microglial activity is implicated in AD. We hypothesize that stimulating M2 microglial phagocytosis specifically decreases plaque load in the AD brain without inducing detrimental immune responses. In this study, the relative contribution of TYROBP and non-TYROBP associated regulators in the internalization of Aβ by microglia is assessed *in vitro* in a BV-2 microglial cell line and in primary mouse microglia. For this purpose, phagocytosis of fluorescent Aβ42 is measured using flow cytometry and microscopy, following reduced or enhanced expression of key players from TYROBP-associated (e.g. TREM2, SIRPβ1 and CR3) and non-TYROBP (e.g. CD14, LRP, RAGE, SRs) receptor signaling cascades. At the same time, the microglial phenotype is determined to check for differentiation towards anti-inflammatory M2 microglia. The most efficient regulators of M2 microglial phagocytosis will be validated *in vivo* in transgenic AD mice.

Disclosures: J. De Vry: None. **P. Vandormael:** None. **T. Vanmierlo:** None. **J. Hendriks:** None. **B. Brône:** None. **J. Prickaerts:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MINECO Grant SAF2013-47989-R

CIBERNED Grant 2013/01

AGAUR Grant 2014SGR1609

Title: FAIM-L controls the shift of TNFalpha-mediated inflammatory response from protection to death

Authors: *J. X. COMELLA[1,2], P. CARRIBA[1], J. URRESTI[1], L. PLANELLS-FERRER[1,2], K. GALENKAMP[1,2], J. VITORICA[5,3], M. GUTIERREZ[6,4], J. LÓPEZ-SORIANO[1,2], B. BARNEDA-ZAHONERO[1,2], N. LLECHA-CANO[1,2];
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Abstract: Neuroinflammation is one of the hallmarks of Alzheimer's disease (AD). Increasing levels of the inflammatory cytokine Tumor Necrosis Factor-alpha (TNFα) have been observed to correlate with disease progression. TNFα has a dual function in neuronal cells: it activates apoptosis by the extrinsic pathway and protects neurons against amyloid-β (Aβ) toxicity. We have examined the actions of a death receptor antagonist, FAIM-L, in the regulation of TNFα roles in AD. FAIM-L protein levels were reduced in the hippocampi of patients with AD, and also in the entorhinal and hippocampal cortex of a mouse model of AD (APP/PS1) before the onset of neurodegeneration. *In vitro*, cultured neurons treated with the cortical soluble fractions of these animals showed a decrease in endogenous FAIM-L, an effect that is also observed by the treatment with Aβ-derived diffusible ligands (ADDLs). The protection afforded by TNFα against Aβ toxicity ceases when endogenous FAIM-L is reduced by short hairpin RNA (shRNA) or by treatment with ADDLs, but FAIM-L protein levels do not modify Aβ toxicity. FAIM-L levels are thus associated with the progression of the neurodegeneration by changing the inflammatory response mediated by TNFα in neurons. We are at present further characterizing this effect in an *in vivo* model of FAIM-L overexpression in neurons by a Tet-Off system.

Disclosures: J.X. Comella: None. **P. Carriba:** None. **J. Urresti:** None. **L. Planells-Ferrer:** None. **K. Galenkamp:** None. **J. Vitorica:** None. **M. Gutierrez:** None. **J. López-Soriano:** None. **B. Barneda-Zahonero:** None. **N. Llecha-Cano:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.19/B106

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FONDECYT Grant 1131025

CONICYT fellowship 21120013

Title: Changes in SR-A expression in aged APP/PS1 mice: their dependence on activation of the TGFβ pathway and their consequence in inflammatory activation of glia

Authors: *F. A. CORNEJO, N. SALGADO, M. ANDRÉS, R. VON BERNHARDI; Pontificia Univ. Católica De Chile, Santiago, Chile

Abstract: Scavenger receptors (SR) are responsible for most of the clearance of cellular debris in the central nervous system. Recent studies have shown that SR not only have a role removing waste from our brain, but they also participate in the inflammatory activation of glial cells. One of the most studied SR is the Class A Scavenger receptor (SR-A), since it has been associated with the pathophysiology of Alzheimer's disease (AD). Here, we show that SR-A expression in microglia and astrocytes of APP/PS1 mice is significantly reduced as animals age, in contrast with the mild increase observed in their wild type counterparts. SR-A expression has been shown to be modulated by the activation of the TGFβ pathway in macrophages, and our group has also reported that TGFβ1-treated microglia had increased expression of SR-A, by a mechanism that is mediated by the transcriptional co-factor Smad3. According to this evidence, in this work we attempted to determine how the TGFβ pathway could modulate SR-A expression in glial cells, and if this cytokine signaling could be related to the reduced expression of SR-A in APP/PS1 aged mice. By using immunofluorescence, here we report that TGFβ1 treatment induces Smad3 activation and its fast translocation to the nucleus in microglia and astrocytes from WT neonatal mice. On the other hand, we observed an altered expression pattern of the TGFβ1 receptor TβRII in APP/PS1 mice by western blot; an effect that could partially explain the impaired expression of SR-A in this animal model. In addition, we developed a triple congenic mouse derived from the transgenic APP/PS1 that overproduces Aβ and mice knockout for SR-A (APP/PS1/SR-A-/-) to analyze the consequences of age-reduced SR-A expression on the behavior and the activation response of the AD mouse model. We have observed altered cytokine profiles in plasma and in the hippocampus of APP/PS1/SR-A-/- mice as they age. Memory impairments were assessed by the Morris water maze neurobehavioral test. Our results suggest that the impairments of the

TGFβ pathway could affect the correct expression of SR-A in the aged APP/PS1 mouse brain, inducing inflammatory and cognitive alterations.

Disclosures: F.A. Cornejo: None. **N. Salgado:** None. **M. Andrés:** None. **R. von Bernhardi:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.20/B107

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Comparison of cell culture systems for the study of microglial biology in Alzheimer's disease

Authors: N. TAUB, E. GROSS, M. FROSCHAUER, *B. HUTTER-PAIER;
QPS Austria Gmbh, Grambach, Austria

Abstract: Besides amyloid plaques and neurofibrillary tangles, chronic inflammation has been identified as another pathological hallmark of Alzheimer's disease (AD). Microglia - brain resident macrophages - are likely to play a key role in the release of inflammatory mediators and in the efficient clearance of amyloid beta, thereby maintaining amyloid plaque homeostasis. Over the years many cell culture systems were developed to examine different aspects of neuroinflammation. In this study, we aimed to compare the advantages and disadvantages of high-through put cell systems as activated murine (BV-2) and human (HMO6) microglial cell lines and more complex systems as stimulated organotypic brain slices by determining cytokine release (TNF-alpha, IL-1beta, IFN-gamma, IL-6, IL-10, KC and IL-12p70), nitrosative stress and amyloid beta clearance. While microglial cell lines allow studying functional aspects of microglia, organotypic brain slices mimic the more complex brain milieu. However, both cell systems are suitable screening tools to identify novel compounds interfering with AD associated inflammation.

Disclosures: N. Taub: None. **E. Gross:** None. **M. Froschauer:** None. **B. Hutter-Paier:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.21/B108

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Novo Nordisk Foundation, grant 12359

Title: Type 2 diabetes mellitus is associated with alterations in astrocyte and neuronal metabolism

Authors: *H. S. WAAGEPETERSEN, J. V. ANDERSEN, M. HOHNHOLT;
Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by an abnormal and persistent increase in blood sugar. A correlation between T2DM and the development of an Alzheimers-like dementia has been shown in animal models and human patients. Impaired cerebral glucose utilization and mitochondrial dysfunction has been reported, but the specific roles of neurons and astrocytes in relation to T2DM remains to be elucidated. In these studies we used a frequently used animal model of T2DM; the db/db mouse. Acutely isolated cortical slices, from 14 week old db/db mice, were incubated with either [U-^{13}C]glucose or [1,2-^{13}C]acetate. Glucose is metabolized by both neurons and astrocytes, whereas acetate is selectively metabolized in astrocytes. The formation of ^{13}C-labeled metabolites was assessed by gas chromatography and mass spectrometry. Preliminary experiments suggest that [U-^{13}C]glucose metabolism was reduced in cortical slices from db/db mice in comparison to control mice. In contrast, the labeling from [1,2-^{13}C]acetate was increased indicating an increased TCA cycle metabolism in astrocytes of cortical slices from db/db mice compared to control mice. Our data suggest an augmentation of mitochondrial metabolism in astrocytes but a general decrease in glucose metabolism in the db/db mouse model of T2DM.

Disclosures: H.S. Waagepetersen: None. **J.V. Andersen:** None. **M. Hohnholt:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant K99 AG044445

NIH Grant P30 AG028383

Title: Microglia heterogeneity in the hippocampus of Alzheimer's disease, dementia with Lewy bodies, and hippocampal sclerosis of aging

Authors: *A. D. BACHSTETTER[1], L. J. VAN ELDIK[2], E. T. IGHODARO[2], E. L. ABNER[2], P. T. NELSON[2];
[1]Sanders-Brown Ctr. on Aging, [2]Univ. of Kentucky, Lexington, KY

Abstract: There is an increasing support for the hypothesis that microglia participate in Alzheimer's disease (AD) pathogenesis. Despite the extensive neuropathological, genetic, and biochemical characterization of microglia in AD little is known about microglial morphology in other common forms of age-related dementia: particularly, dementia with Lewy bodies (DLB) and hippocampal sclerosis of aging (HS-Aging). The clinical disease formerly referred to simply as "Alzheimer's disease" is, at the population level, a complex manifestation of many different brain conditions. These age-related brain pathologies include AD (characterized by amyloid plaques and neurofibrillary tangles), as well as cerebrovascular disease, DLB, and HS-Aging. Here we studied cases with pathologically-confirmed AD (n=7), HS-Aging (n=7), AD + HS-aging (n=4), DLB (n=12), and normal (cognitively intact) controls (NC) (n=9) from the University of Kentucky Alzheimer's Disease Center autopsy cohort. The Aperio ScanScope digital neuropathological tool was used along with two well-known microglial markers: IBA1 (a marker for both resting and activated microglia) and CD68 (a lysosomal marker in macrophages/microglia associated with phagocytic cells). Hippocampal staining analyses included studies of subregions within the hippocampal formation and nearby white matter. In addition, we defined and quantified, in the CA1 region, five microglia morphological phenotypes in the autopsy samples: ramified, hypertrophic, dystrophic, rod-shaped, and amoeboid. Using these tools and methods, we describe variation in microglial characteristics that show some degree of disease specificity, including, (1) increased microglia density and number in HS-aging and AD + HS-aging; (2) low microglia density in DLB; (3) increased number of dystrophic microglia in HS-aging; and (4) increased proportion of dystrophic to all microglia in DLB. We conclude that variations in morphologies among microglial cells, and cells of macrophage lineage, can help guide future work connecting neuroinflammatory mechanisms with specific neurodegenerative disease subtypes.

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Poster

039. Neuroinflammation and Alzheimer's Disease

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Title: Trem2 has age-dependent effects on myeloid cell activation and pathology in Alzheimer's disease mouse models

Authors: *T. R. JAY[1], S. BEMILLER[2], A. HIRSCH[1], M. BROIHIER[1], C. MILLER[2], B. T. LAMB[2], G. E. LANDRETH[1];
[1]Neurosciences, Case Western Reserve Univ., Cleveland, OH; [2]Neurosci., Cleveland Clin. Lerner Res. Inst., Cleveland, OH

Abstract: Alzheimer's disease (AD) is characterized by aberrant neuroinflammation. The integral role of inflammation in AD pathogenesis was highlighted by studies which identified variants in Trem2, an important modulator of myeloid cell activation, which confer high risk for developing AD. We have recently shown that Trem2 deficiency reduces myeloid cell activation and accumulation around plaques and ameliorates AD pathologies in 4-month-old APPPS1-21 mice. However, others have shown that Trem2 increases pathology in 8-month old 5XFAD mice. One possible explanation for these divergent findings is that Trem2 has differential affects early and late in pathology. Here we assess whether Trem2 has an age-dependent role in myeloid cell activation and amyloid pathology by examining 2- and 8-month-old APPPS1;Trem2-/- mice. We also examine possible mechanisms by which Trem2 deficiency prevents myeloid cell activation and accumulation. We further confirm that Trem2 expressing cells are peripherally-derived using radiation chimeras, and examine proliferation and cell death of resident and peripherally-derived myeloid cell populations in Trem2 deficient APPPS1 mice. These data further our understanding of how Trem2 loss affects myeloid cell function, which will be important to more fully understand the role of inflammation in AD.

Disclosures: **T.R. Jay:** None. **S. Bemiller:** None. **A. Hirsch:** None. **M. Broihier:** None. **C. Miller:** None. **B.T. Lamb:** None. **G.E. Landreth:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Instituto de Salud Carlos III and FEDER (European Union)

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Junta de Andalucia, Consejeria de Economia, Innovacion, Ciencia y Empleo, Proyectos de Excelencia (P11-CTS-7847).

Title: Diabetes mellitus induces vascular damage, inflammation and neuronal loss in a murine model of Alzheimer’s disease

Authors: *J. RAMOS RODRÍGUEZ, C. INFANTE-GARCIA, L. GALINDO-GONZALEZ, M. GARCIA-ALLOZA;
Sch. of Medicine. Univ. of Cadiz, Cadiz, Spain

Abstract: Alzheimer’s disease (AD) and vascular dementia (VaD) are the most common forms of dementia, clinically characterized by cognitive decline. Whereas the ultimate causes remain largely unknown, many studies support the relationship between AD-VaD and metabolic disorders. Among these, diabetes mellitus (DM) seems to be of special relevance and it is a risk factor to develop dementia, although the mechanisms implicated have not been elucidated. In order to further explore the relationship between DM and AD-VaD, as well as the effects of a mixed pathology on the central nervous system we induced hyperglycemia in APP/PS1 mice. Animals were i.p. treated with streptozotocin (STZ), 40 mg/Kg for 5 consecutive days, at 4 months of age. Mice were then aged up to 6 months, when AD features are already established in this mouse model. At 6 months of age, STZ-wildtype and STZ-APP/PS1 mice showed high glucose and low insulin plasma levels, as typical metabolic alterations observed in type 1 DM. Both, widltype and AD mice, treated with STZ presented severe brain atrophy, that preferentially affected the cortex, while the hippocampus was spared. Further inmunohystochemical studies revealed significant neuronal loss in STZ-treated mice, that worsened in the proximity of senile

plaques in case of APP/PS1-STZ animals. Microglia activation, indicative of the inflammatory processes, was significantly increased in all STZ-treated mice, and this effect was worsened in APP/PS1-STZ animals. To deepen in the study of central disorders, we used Prussian blue staining to detect spontaneous hemorrhages. We observed that cortical hemorrhage burden was increased in STZ mice, and significantly worsened in APP/PS1-STZ mice. These data suggest a synergistic effect of APP/PS1 transgenes and STZ treatment, and it remains feasible that increased bleeding in APP/PS1 mice may underlie observed neuronal loss. Altogether, our data support a direct relationship between DM and brain atrophy, neuronal loss and vascular damage. DM might be more than a risk factor to develop dementia, and synergistically contribute to the degenerative process observed in AD and VaD. Our data provide new insights into the causes of late-life AD and may set the basis to develop novel strategies to prevent dementia.
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Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.25/B112

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA AG12411

Windgate Foundation

Title: Interleukin 1: A major player in Alzheimer-related autophagic dysfunction

Authors: *P. A. PARCON[1], L. LIU[2], R. A. JONES[2], R. E. MRAK[3], W. T. GRIFFIN[2]; [1]RIOA, [2]Reynolds Inst. on Aging, UAMS, Little Rock, AR; [3]Dept. of Pathology, Univ. of Toledo Hlth. Sci. Campus, Toledo, OH

Abstract: Neuroinflammation is recognized as a major player in Alzheimer (AD) pathogenesis. Here we extend its influence to include autophagy, in particular with regard to APOE genotype. Firstly, we report that the AD-related nuclear-to-cytoplasmic translocation of NEDD8 in

hippocampal neurons is mimicked by treatment of primary rat neurons treated with IL-1β. Secondly, we show in the PD-APP mouse model of AD that an elevation of IL-1α and IL-1β mRNA at 2 months of age - one month prior to cognitive deficits and 4-7 months before plaque deposition - that is even greater at 17 months, and is accompanied by a marked elevation in the E3 ubiquitin-ligase parkin. This, together with the increase in colocalization of parkin and NEDD8 in primary rat neurons treated with IL-1β and the increase in both total parkin protein and NEDD8-conjugated parkin in NT2 cells treated with IL-1β, suggests that IL-1β facilitates parkin-dependent ubiquitination via upregulation of both parkin protein and parkin neddylation. Thirdly, we find similar relationships between the levels of IL-1 and autophagy-related mRNAs in hippocampal tissue homogenates from AD compared to age-matched control patients. Moreover, such changes were influenced by APOE genotype with an increasing trend in protein levels of autophagy substrate SQSTM1/p62: AMC 3,3 < AD, and AD 3,3 < AD 3,4< AD 4,4. Interestingly, while mRNA levels of p62, LC3, and LAMP2 did not differ between AD 4,4 carriers and AMC 3,3 carriers, those with AD 3,3 showed a marked elevation, suggesting that inheritance of APOE ε4 alleles is associated with a weakened autophagy system in which p62 protein levels build up despite transcriptional down-regulation. Taken together with previous reports of elevated expression of IL-1α and β in AD, the data reported here is consistent with the idea that IL-1β is a major player in protein degradation pathways associated with Alzheimer pathogenesis. Supported in part by NIA AG12411 and the Windgate Foundation.

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Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.26/C1

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Restoration of the peripheral immune/inflammatory response correlates with brain injury recovery in a murine model of Alzheimer’s disease

Authors: G. DI BENEDETTO, G. FUCCIO SANZÀ, G. CANTARELLA, *R. BERNARDINI; Univ. Catania Sch. of Med., Catania, Italy

Abstract: Alzheimer disease (AD) related inflammation is triggered concomitantly with deposition of amyloid beta in the brain and is characterized by increased expression of inflammatory/immune mediators, including cytokines belonging to the TNF superfamily such as

TNF-Related-Apoptosis-Inducing-Ligand (TRAIL). Accumulation of activated microglia producing TNF-α and MCP has been reported either in AD animal models as well as in the human AD brain. However, little is known about changes occurring in peripheral immune response in AD. Here, we investigated the inflammatory/immunological abnormalities occurring in the spleen of a murine model of AD, and whether treatment with a TRAIL neutralizing antibody, known to exert neuroprotective effects in similar animal model, could also be related to improvement of peripheral inflammatory/immunological parameters. The effects of TRAIL neutralization on the morphology of the spleen as well as the expression of TRAIL, DR5, and inflammatory mediators were studied in 3xTg-AD mice. Treatment with the TRAIL neutralizing antibody resulted in improvement of morphology of spleens, as well as in decreased expression of inflammatory molecules. Finally, neutralization of TRAIL resulted in restrained peripheral immune/inflammatory response, which correlates with CNS functional and tissue parameters of 3xTg-AD mice. Thus, it is plausible to propose the peripheral immune system as a target for innovative treatment of neuroinflammatory disorders.

Disclosures: G. Di Benedetto: None. **G. Fuccio Sanzà:** None. **G. Cantarella:** None. **R. Bernardini:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.27/C2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CAL Grant - Roma Tre University

MIUR Grant - PhD Fellowship

Title: Oxidative damage and antioxidant response during the progression of Alzheimer’s pathology in Tg2576 mouse neocortex

Authors: A. FRACASSI[1,2], G. TAGLIALATELA[2], *S. MORENO[1];
[1]Univ. Roma Tre, Rome, Italy; [2]Univ. of Texas Med. Br., Galveston, TX

Abstract: Alzheimer’s Disease (AD) is the most common form of dementia, characterized by progressive neurodegeneration of cortical structures. Neuropathological changes include the formation of extracellular senile plaques and intracellular neurofibrillary tangles, followed by inflammation and massive neuronal loss [1]. The mechanisms underlying AD pathogenesis,

triggered by amyloid beta (Aβ) peptide accumulation, include oxidative stress, deriving from deregulation of energy homeostasis and involving mitochondria and peroxisomes [2,3]. We here addressed the oxidative stress status and the elicited cellular response at the onset and during the progression of Aβ pathology. As a suitable model for this purpose, and consistent with our previous investigations, we chose the Tg2576 mouse model [4], displaying a slowly progressive Aβ pathology. We focussed on the neocortex of mice aging 3, 6, 9, 12, and 18 months to study age-dependent changes of oxidative modification markers, antioxidant enzymes and related transcription factors. The expression of these molecules was analysed in relation to the distribution of Aβ peptide and oligomers, by a combined molecular/morphological approach. Nucleic acid oxidative damage in the cytoplasm and mitochondria, accompanied by defective antioxidant defences and decreased PGC1α expression are already detected in 3-month-old Tg2576 neurons. Conversely, peroxisome proliferator-activated receptor α is increased in these cells. The predominantly cytoplasmic immunostaining found in Tg neurons possibly reflects a nongenomic action of this redox sensor molecule, leading to inhibition of inflammatory gene expression. At 6 months, concominant with Aβ intracellular accumulation, PMP70 is downregulated, indicating impaired peroxisomal translocation, and consequent harmful accumulation, of fatty acids. In 9-month-old Tg neocortex, Aβ oligomers and acrolein deposition positively correlates with GFAP, GPX1 and PMP70 increases, supporting a compensatory response, involving astroglial peroxisomes. At severe AD stages, when senile plaques disrupt cortical cytoarchitecture, antioxidant capacity is gradually lost. Overall, our study suggests that therapeutic intervention targeting peroxisomes in AD should be considered. References 1.Querfurth, HW, LaFerla, FM. NEJM, 362: 329, 2010. 2.Sultana, R, Butterfield, DA. JAD, 19: 341, 2010. 3.Fanelli, F, Sepe, S, D'Amelio, M, Bernardi, C, Cristiano, L, Cimini, A, Cecconi, F, Ceru', MP Moreno, S. Mol Neurodegener, 8: 8, 2013. 4.Hsiao, K, Chapman, P, Nilsen, S, Eckman, C, Harigaya, Y, Younkin, S, Yang, F, Cole, G. Science 274: 99, 1996.

Disclosures: **A. Fracassi:** None. **G. Taglialatela:** None. **S. Moreno:** None.

Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.28/C3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: IBRO International Travel Grants

Title: Type I and II interferon responses are altered in the choroid plexus and in the hippocampus of a mouse model of Alzheimer's disease

Authors: S. D. MESQUITA[1], A. C. FERREIRA[1], *A.-J. RODRIGUES[1], F. GAO[2], G. COPPOLA[2], D. H. GESCHWIND[2], J. C. SOUSA[1], M. CORREIA-NEVES[1], N. SOUSA[1], J. A. PALHA[1], F. MARQUES[1];
[1]Univ. of Minho, Life and Hlth. Sci. Res. Inst. (ICVS), Braga, Portugal; [2]Program in Neurogenetics, Dept. of Neurology, David Geffen Sch. of Med. - Univ. of California, Los Angeles, CA, USA., Los Angeles, CA, CA

Abstract: Alzheimer's disease (AD) is a neurodegenerative disease characterized by a marked decline in cognition and memory function. One of the major pathological hallmarks of AD is the progressive accumulation and deposition of amyloid beta (Aβ) peptides in the brain. Importantly, increased Aβ toxicity and pathology is accompanied by alterations in parallel mechanisms and responses that regulate brain homeostasis and function. Increasing evidence highlights the essential role of neuroinflammatory and immune-related molecules, including those produced at the brain barriers, on brain immune surveillance, decreased Aβ clearance from the brain and increased brain cell dysfunction and pathology in AD. Therefore, understanding the response at the brain barriers may unravel novel pathways of relevance for the pathophysiology of AD. Herein, we focused on the study of the choroid plexus (CP), which constitutes the blood-cerebrospinal fluid barrier, in aging and in AD. Specifically, we used the PDGFB-APPSwInd (J20) transgenic mouse model of AD, which presents early memory decline and progressive Aβ accumulation in the brain, and littermate age-matched wild-type (WT) mice, to characterize the CP transcriptome at 3, 5-6 and 11-12 months of age. The most striking observation was that the CP of J20 mice displayed an overall overexpression of type I interferon (IFN) response genes at all ages. Moreover, J20 mice presented a high expression of type II IFN genes in the CP at 3 months, which became lower than WT at 5-6 and 11-12 months. Importantly, along with a marked memory impairment and increased glial activation, J20 mice also presented a similar overexpression of type I IFN genes in the dorsal hippocampus at 3 months. Altogether, these findings provide new insights on a possible interplay between type I and type II IFN responses in AD and point to IFNs as targets for modulation in cognitive decline.

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Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.29/C4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: State of Texas Norman Hackerman Advanced Research Program

Title: Repeated exposure to poly i:c leads to elevations in hippocampal amyloid-beta, cognitive dysfunction, and sustained deficits in burrowing

Authors: *J. D. WHITE[1], M. J. EIMERBRINK[2], A. D. HARDY[3], D. KRANJAC[4], K. C. PAULHUS[3], G. W. BOEHM[2], M. J. CHUMLEY[3];
[1]Dept. of Psychology, [2]Psychology, [3]Biol., Texas Christian Univ., Fort Worth, TX; [4]Psychiatry and Behavioral Sci., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Current global statistics estimate that 44.4 million people are afflicted with dementia, and that 50–75% of these patients suffer from Alzheimer's disease (AD), a progressive disorder categorized by neuronal and behavioral deterioration (Prince et al. 2013). One hallmark of AD neuropathology is the accumulation of the amyloid-beta (Aβ) peptide, that is known to inhibit cell-to-cell communication, and lead to cognitive deficits and neuronal cell death. Our lab has previously shown that repeated bouts of peripheral inflammation are sufficient to significantly increase the amount of Aβ in the hippocampus of treated animals, and cause deficits in cognition. The goal of the present research was to extend the duration of inflammatory immune challenges to monitor the corresponding changes in sickness behaviors, central Aβ accumulation, and cognitive deficits induced by said Aβ accumulation. All experiments utilized polyinosinic:polycytidylic acid (poly I:C), a viral mimetic, as an immune challenge to induce an inflammatory response, and subjects were compared to saline treated controls. We tested the influence of daily injections of poly I:C, over a 21 day period, on sickness behaviors, using a burrowing paradigm. Results indicate that the inflammatory response to poly I:C over this duration is sufficient to consistently induce sickness behaviors across all days of testing. Next, we examined the effect of poly I:C on cognitive deficits and central Aβ accumulation, over three treatment durations: 7, 14, and 21 days. Forty-eights hours after the final injection, animals were trained in a contextual fear conditioning paradigm, and tested 24 hours later. The hippocampus was then collected and Aβ levels were quantified. Results show that poly I:C can disrupt cognition after both 14 and 21 days of administration, while at all time points, a significant elevation in Aβ within the hippocampus was found. These results demonstrate that peripheral injections of poly I:C are a reliable means to induce sickness behaviors over an extended duration. Moreover, repeated poly I:C exposure is capable of inducing a significant elevation in central Aβ after 7 days, which can be maintained with consistent injections. Finally, we identified that Aβ accumulation can disrupt cognition after 14 and 21 days, but not after 7 days, despite a significant increase in Aβ. This may indicate the presence of a physiological threshold for Aβ that must be exceeded to disrupt cognition. In addition, these results suggest that long

term administration of poly I:C may provide a model to investigate the effects that chronic inflammation has on the development of Alzheimer's-like pathology.

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Poster

039. Neuroinflammation and Alzheimer's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 39.30/C5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Tau oligomers co-localize with inflammatory markers in Frontal Temporal Lobe dementia

Authors: *A. N. NILSON[1], K. ENGLISH[2], J. E. GERSON[1], R. KAYED[3];
[1]Neurosci. and Cell biology, [2]Med., [3]Neurol., Univ. of Texas Med. Br., Galveston, TX

Abstract: Inflammation plays a large role in age-related diseases including tauopathies such as Frontal Temporal Lobe dementia (FTLD). Neurodegenerative diseases exhibit inflammation and cell death before larger aggregates form. Tau aggregates in neurodegenerative disease into large aggregates called neurofibrillary tangles. However, smaller soluble aggregates -oligomers-are formed prior to tangles and evidence suggests that they are actually the most toxic species. Thus we decided to investigate a connection between tau oligomers and inflammation. Human cortex samples from control and FTLD brains were sectioned. The sections underwent immunohistochemistry using two fluorescent stains and DAPI nuclei stain. The antibodies included both oligomer-specific and total tau, as well as various inflammation markers such as GFAP (astrocyte marker), Iba1 (microglia marker), and HMGB1 (inflammation marker). Additionally, homogenates were prepared for ELISA to determine levels of inflammatory proteins. There was co-localization seen with tau and several of the inflammation markers. GFAP showed changes in number of cells and morphology. T22 also co-localized with Iba1 and HMGB1. The ELISA showed an increase in GFAP. Oligomeric tau co-localizes with inflammatory markers indicating it may stimulate the neuroinflammation seen in FTLD. These preliminary results indicate oligomeric tau is associated with the inflammatory response in FTLD subjects. Further investigation of this response to oligomeric tau could provide insight for developing treatments for FTLD. Furthermore, oligomeric tau plays a role in other diseases such as Alzheimer's and Parkinson's and deserves further investigation. Anti-inflammatory drugs may play an important role in the treatment of these diseases by decreasing the inflammation and cell death due to oligomeric tau.

Disclosures: **A.N. Nilson:** None. **K. English:** None. **J.E. Gerson:** None. **R. Kayed:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.01/C6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Live charitable foundation

Joseph Sagol postdoctoral fellowship

Title: m6a regulatory proteins in human Alzheimer's disease neurons

Authors: *A. LICHT-MURAVA[1], S. ILYAS[1], P. KATSEL[1], P. ROUSSOS[1], M. BERRI[2], G. RECHAVI[2], V. HAROUTUNIAN[1];
[1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Sheba Med. Ctr., Ramat Gan, Israel

Abstract: Epigenetic modification of RNA is another layer of regulation between DNA and protein translation. N(6)-methyladenosine (m^6A) is one of the most common RNA modifications. A Methyltransferase complex for m^6A, containing: methyltransferase like 3 (METTL3) and methyltransferase like 14 (METTL14) proteins, has been identified. In addition, two demethylases: Fat mass and obesity-associated protein (FTO), and AlkB family member 5 (ALKBH5) have been shown to dynamically regulate m^6A. A growing body of evidence is beginning to identify specialized cell-type specific alteration and regulation of gene and protein expression in health and disease. Human brain function depends not only on different cell types but also on contacts between them. The current study aimed to determine if the regulation of m^6A changed in different brain cell types and whether these changes exist in Alzheimer disease (AD). Laser capture microdissection was used to isolate endothelial cells and neurons near blood vessels from the hippocampus of healthy individuals and those with varying severities of AD. This study shows that FTO, ALKBH5, METTL3 and METTL14 were abundant specifically in neurons and were decreased significantly with AD severities. Moreover, total RNA and protein expression show significant decrease of FTO levels in the superior temporal cortex of individuals with different degrees of dementia or different severities of AD associated neurofibrillary (NFT) pathologies (Braak stage >0). The most significant decrease in FTO RNA and protein levels were in the first stages of neurofibrillary tangle pathology development where NFTs are confined to the transentorhinal region. Studies have shown that FTO decrease leads to m^6A increase and that m^6A modifications contribute to the regulation of translation and lifetime of mRNA.

Collectively, this study suggests that epigenetic regulation of RNA expression in neurons may play a significant role in the development of dementia and AD.

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Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.02/C7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Association of Alcadein alpha with kinesin light chain is regulated by phosphorylations at cytoplasmic acidic region

Authors: *Y. SOBU, S. HATA, T. SUZUKI;
Grad. Sch. of Pharm.Sci. Hokkaido Univ., Sapporo/Hokkaido, Japan

Abstract: Alcadeins(Alc) / Calsyntenins are type I membrane proteins consisted of Alcα, Alcβ and Alcγ, which express predominantly or exclusively in neuron (J. Biol. Chem. [2003] 278, 49448). Alcs are found as molecules suppressing the cleavage of amyloid precursor protein (APP) by forming a complex with cytoplasmic X11L adaptor protein. As is APP, Alcs are primarily cleaved by ADAM10/17 at jaxtamembrane region and then subject to the intracellular cleavage by γ-secretase (J. Biol. Chem. [2004] 279, 24343). This is mentioned as the regulated intramembrane proteolysis (RIP) to release intracellular cytoplasmic domain (ICD) fragment into cytoplasm (J. Biol. Chem. [2009] 284, 36024). Moreover, Alcα is known to function as a cargo receptor of kinesin-1. Kinesin-1, a heterotetramer composed of two heavy chains (KHC) and two light chains (KLC), is an anterograde molecular motor transporting cargos toward nerve terminus. Alcα binds to KLC through the WD motif in the cytoplasmic region. This binding activates kinesin-1, then vesicles containing Alcα are transported from cell body to nerve end in axon (EMBO J. [2007] 26, 1475; Traffic [2012] 13, 834). However, it remains unclear how Alcα vesicular transport is regulated. Here, we focused our analysis on the regulation of Alcα association with kinesin-1. We found that the cytoplasmic domain of Alcα includes many consensus sequences for phosphorylation, especially by casein kinase I and/or II. Because we found that the cytoplasmic sequence of Alcα is phosphorylated in brain *in vivo*, we investigated the function of Alcα phosphorylation in the interaction with kinesin-1. Alcα-FLAG expressed in N2a cell are recovered with anti-FLAG antibody and dephosphorylated with λPPase. The dephosphorylated Alcα-FLAG was mixed with N2a cell lysate expressing HA-KLC1 to examine

a binding ability to KLC. Dephosphorylation of Alcα-FLAG decreased the binding with HA-KLC1. Then we tried to determine the phosphorylable residues which regulate the interaction with KLC by the procedures using deletion constructs and the alanine-scanning mutagenesis into the candidate sites. We detected the phosphorylation sites in the acidic region of Alcα. These results suggest that transport of Alcα vesicle by kinesin-1 is regulated by phosphorylation of Alcα intracellular domain.

Disclosures: **Y. Sobu:** None. **S. Hata:** None. **T. Suzuki:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.03/C8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grand Valley State University Presidential Research Grant

Title: Assessment of Parkinson’s disease specific microRNA in Alzheimer’s disease

Authors: *S. MUKHOPADHYAY;
Grand Valley State Univ., Grand Rapids, MI

Abstract: Alzheimer’s Disease (AD) and Parkinson's Disease (PD) are distinct conditions. However, mounting evidence shows possible links between the genetics and brain changes associated with them such as cognitive impairment and aggregation of misfolded proteins, suggest cross disease association. Previous research has identified a panel of plasma based PD specific microRNA biomarkers. The pathway analyses of these microRNAs target genes showed enrichment in the neuron differentiation/projection and synaptic pathways, which are also important signaling pathways in AD. Here we find novel evidence that shows the presence of PD specific microRNA in AD brain samples. There was up regulation of all microRNA in mid stage and late stage of AD samples compared to the healthy control samples. In general, inferior temporal cortex of hippocampus showed higher microRNA expression compared to the frontal cortex of the same patient. We identified that microRNA cross-talk is present between PD & AD. This study is essentially an important first step towards future functional studies of microRNA analyses for potential therapeutic targets for AD.

Disclosures: **S. Mukhopadhyay:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.04/C9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS KAKENHI 25670425

Title: A role of aquaporine-4 in immune responses in Alzheimer's disease model mice

Authors: *T. NIIKURA[1], H. WADA[1], M. YASUI[2], Y. ABE[2];
[1]Sophia Univ., Tokyo, Japan; [2]Dept. of Pharmacol., Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: Astrocytes have essential roles in maintaining stable physiological conditions in the brain. In addition, astrocytes are implicated in specific neuronal functions such as the facilitation of learning and memory. Aquaporin-4 (AQP4) is the major water channel in the CNS and enriched in the subpial and perivascular endfeet of astrocytes. The AQP4 expression is up-regulated in the injured brain and the astrocytes migrate to the injured site in an AQP4-depepndent manner. In the AQP4-deficient (AQP4/KO) mice, the up-regulation of pro-inflammatory cytokines induced by brain injury is significantly attenuated, suggesting that AQP4 has an essential role in immunological function under pathological conditions in the CNS. Multiple studies have demonstrated that inflammatory cytokines are up-regulated and reactive astrocytes and activated microglia accumulate surrounding the amyloid plaques in brains of Alzheimer's disease (AD) patients and AD model mice. To clarify the effect of astrocyte dysfunction in the AD pathogenesis, we established AQP4-deficient AD mouse model (AQP4/KO-5xFAD) with C57BL/6J background by crossing AQP4/KO mice and amyloid overproducing AD model mice, B6-Cg-Tg (APPSwFlLon,PSEN1*M146L*L286V)6799Vas (5xFAD). At six months of age, behavioral tests were performed and brains were subjected to the immunohistological analysis. In the behavioral tests, no significant difference was observed between 5xFAD and AQP/KO-5xFAD female mice in both open field and Y-maze tests. Contrary to our expectation, amyloid plaque formation or reactive astrocytosis was not altered by AQP4 deficiency. However, microgliosis was significantly attenuated in the AQP4/KO-5xFAD mice as compared with 5xFAD mice. These findings suggest that AQP4 is involved in the induction of microgliosis underlying AD pathogenesis.

Disclosures: **T. Niikura:** None. **H. Wada:** None. **M. Yasui:** None. **Y. Abe:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.05/C10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NMRC Singapore grant

Title: Differential associations between retinal ganglion cell thickness and white matter integrity among healthy normal, cognitive impairment, and Alzheimer's disease

Authors: *S. LIU[1], Y. ONG[3], S. HILAL[2], Y. LOKE[1], C. CHEN[2], C. CHEUNG[3], J. ZHOU[1]; [1]Ctr. for Cognitive Neuroscience, Neurosci. and Behavioral Disorder Program,, [2]Dept. of Pharmacol., Natl. Univ. of Singapore, Singapore, Singapore; [3]Singapore Eye Res. Inst., Singapore Natl. Eye Ctr., Singapore, Singapore

Abstract: Introduction: Alzheimer's disease (AD) is associated with disrupted brain white matter integrity [1]. Recently, our group reported thinning of macular ganglion cell-inner plexiform layer (GC-IPL) measured by spectral-domain optical coherence tomography (SD-OCT) in patients with AD and mild cognitive impairment [2]. However, the link between GC-IPL thickness and brain white matter integrity in normal and pathological ageing remains unknown. Here, using SD-OCT and Diffusion Tensor Imaging (DTI) techniques in subjects with no cognitive impairment (NCI), cognitive impairment without dementia (CIND) and AD, we hypothesized that a thinner GC-IPL, reflecting retinal ganglion cell loss in retina, would be associated with reduced white matter integrity. **Methods:** 180 subjects (65 NCI, 68 CIND, and 47 AD) underwent DTI scans (Siemens Tim Trio, 3T, 61 diffusion directions at b=1150 s/mm^2 and 7 b0 maps) and SD-OCT (Cirrus OCT, Carl Zeiss Meditic) scans. After retina image quality control, 124 subjects remained (47 NCI, 50 CIND, and 27 AD). After standard preprocessing using TBSS [3], group differences in fractional anisotropy (FA) and mean diffusivity (MD) were examined across the three groups using pairwise two-sample t-tests. Whole-brain skeletonized voxelwise regression of two DTI indices (FA and MD) against GC-IPL thickness performed across and within each of the groups. All analyses were controlled for age, gender, handedness, and ethnicity. **Results:** We observed that white matter integrity deteriorated (evidenced by FA decrease and MD increase) as with increasing severity of cognitive impairment from NCI to CIND and AD (FWE corrected $p<.005$). Furthermore, the GC-IPL thickness was correlated with FA positively and MD negatively (FWE corrected $p<.05$) in NCI, mainly in anterior thalamic radiations, corticospinal tracts, cingulum cingulated and hippocampus, forceps minor, inferior fronto-occipital fasciculus, and superior longitudinal fasciculus. At the same threshold, no correlations were found in CIND or AD. However, there was a trend of positive (negative)

correlation between FA (MD) in the fornix and the cingulum cingulate and GC-IPL thickness (uncorrected p<.05) for the CIND, and positive correlation between FA in the fornix and the GC-IPL thickness for the AD. **Conclusion:** Our findings suggest that thinning GC-IPL may be associated with deterioration of white matter integrity in normal aging. Though AD affects both retinal ganglion cell and white matter integrity, the correlation between these markers are altered, suggesting a disruption of normal physiological age-related relationships due to disease-induced changes.

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Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.06/C11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01NS075487

Title: A role for the Alzheimer’s disease risk factor CD2AP in mediating blood-brain barrier integrity in mice

Authors: ***J. COCHRAN**, T. RUSH, S. C. BUCKINGHAM, E. D. ROBERSON;
Ctr. for Neurodegeneration and Exptl. Therapeut., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: CD2-associated protein (CD2AP) is a leading genetic risk factor for Alzheimer’s disease, but little is known about the function of CD2AP in the CNS. We used CD2AP -/- mice to determine the role of CD2AP in the CNS. Because CD2AP -/- mice normally die by 6 weeks of age from nephrotic syndrome, we used mice that also express a CD2AP transgene in the kidney, but not brain, to attenuate this phenotype. We found that CD2AP-deficient mice did not exhibit abnormalities in behavior, including learning and memory, except for motor deficits in a subset of CD2AP -/- mice exhibiting severe nephrotic syndrome. This suggests that CD2AP does not overtly affect behavior in mice, and that the motor deficits we observe in a subset of CD2AP -/- mice are due to systemic illness. We also observed more severe pentylenetetrazol-induced seizures in CD2AP -/- mice, but characteristics of these seizures on EEG were not altered. As CD2AP is known to be expressed in brain-adjacent endothelial cells, we speculated that the increased susceptibility to seizures without detectably different seizure characteristics may be

due to increased availability of pentylenetetrazol due to compromised blood-brain barrier integrity. Using sodium fluorescein extravasation, we found that CD2AP -/- mice had reduced blood-brain barrier integrity, suggesting that increased seizure susceptibility in these mice may depend simply on the availability of pentylenetetrazol. Neither seizure measures nor blood-brain barrier integrity were correlated with a marker of nephrotic syndrome, suggesting that these measures are dissociable from the systemic illness present with CD2AP -/-. Taken together, our results suggest a new role of CD2AP in mediating blood-brain barrier integrity and indicate that further investigation of non-neuronal roles of CD2AP relevant to the CNS is justified.

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Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.07/C12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HKU Alzheimer's Disease Research Network

HKU Seed Funding for Basic Science Research (201311159171)

generous donation from Ms. Kit Wan Chow

Title: Proteasome and autophagic degradation in an *in vitro* model of Alzheimer's disease

Authors: *R. C. CHANG[1,2], S. S. Y. CHENG[1], C. H. L. HUNG[1];
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Abstract: Alzheimer's disease (AD) is the leading cause of dementia, affecting about 44 million people worldwide and amounting to over $605 billion in healthcare costs. Therefore, it is important to understand the initiation and pathogenesis of AD in hopes of finding a cure. Since AD is classified by the accumulation of β-amyloid (Aβ) and hyperphosphorylated tau protein, it has been speculated that impairments in key protein degradation systems such as the ubiquitin-proteasome system (UPS) or autophagy-lysosomal pathway (ALP) may be important contributors to AD pathology. Also, both of these pathways have been found to be affected in postmortem brain of AD patients. Therefore, we hypothesize that dysfunctions in the UPS and

ALP play an important role in AD pathogenesis. Using primary culture of cortical neurons treated with oligomeric Aβ as an *in vitro* model of AD, biochemical and imaging techniques were employed to investigate changes in UPS and ALP activity after exposure to Aβ peptide. Our data showed an initial impairment proteasome activity followed by an induction of autophagy at later time points. Different isoforms of tau were found to accumulate and aggregate at different time points in neurons exposed to Aβ where protein degradation impairments were found. The accumulation in phosphorylated tau was not due to changes in kinase or phosphatase activity but likely due to impairments in protein degradation signaling. Consequences of impaired protein degradation in neurons were also investigated by examining changes in synaptic integrity and function. As tau is a microtubule stabilizing protein, impairments in tau turnover after exposure to Aβ peptide will affect microtubule stability, resulting in disturbances in axonal transport and synaptic function, ultimately resulting in neuronal loss. Therefore, the effects of tau accumulation and phosphorylation on synaptic protein expression and function were examined. To date, our data suggest that the UPS is affected at early time points, resulting in the accumulation and subsequent aggregation of tau within neurons exposed to Aβ peptide. The presence of tau aggregates initiates the ALP to promote the removal of these aberrant proteins, to bring neurons back to protein homeostasis. With the accumulation and aggregation of tau, other factors contributing to neuronal cell death such as a loss in synaptic protein expression may also be affected

Disclosures: **R.C. Chang:** None. **S.S.Y. Cheng:** None. **C.H.L. Hung:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.08/C13

Topic: C.02. Alzheimer's Disease and Other Dementias

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Fundacion Eugenio Rodriguez Pascual 2015

Junta de Andalucia, Consejeria de Economia, Innovacion, Ciencia y Empleo, Proyectos de Excelencia (P11-CTS-7847)

Title: Long-term brain pathology in a mixed model of Alzheimer’s disease and type 2 diabetes

Authors: *C. INFANTE GARCIA, J. RAMOS-RODRIGUEZ, A. GARCIA-ALCINA, M. GARCIA-ALLOZA;
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Abstract: Alzheimer's disease (AD) is the leading cause of dementia worldwide. Neuropathological features include: 1) neurofibrillary tangles with abnormally phosphorylated tau protein, 2) senile plaques (SP), mainly composed of amyloid-beta peptide (Aβ), and 3) neuronal loss. Whereas aging remains the main risk factor to suffer dementia, hyperinsulinemia and type 2 diabetes mellitus (T2D) are also relevant risk factors to develop dementia. Since the EA and DM2 are chronic diseases closely associated with aging, we have explored this relationship in the long term. We have characterized a mixed model of T2D and AD, resulting from crossing an AD model (APP/PS1 mice) with a classical model of T2D, the db/db mouse. Pathological hallmarks were assessed at 36 weeks of age, when both pathologies have chronified. In APP/PS1xdb/db mice body weight, postprandial blood glucose and insulin levels were followed at 4, 18 and 36 weeks of age. Our studies revealed an overall increase of glucose levels in APP/PS1xdb/db mice. On the other hand, insulin levels were reduced with aging, showing premature exhaustion of beta-pancreatic cells in the mixed model. Learning and memory were assessed by the Morris water maze and new object discrimination tests and cognitive compromise observed in APP/PS1 and db/db animals was significantly worsened in APP/PS1xdb/db mice. Postmortem studies revealed a shift in Aβ profile, and whereas insoluble Aβ was reduced in our mixed model, soluble more toxic species were significantly favoured. We also observed that tau phosphorylation was increased in APP/PS1xdb/db mice. Central atrophy was detected in db/db mice and a worsening effect was observed in the APP/PS1xdb/db group. Further the assessment of dendritic complexity revealed a relevant compromise in diabetic mice, that was worsened in the APP/PS1xdb/db mice. Altogether, our data suggest that T2D and AD have a synergistic effect, both at metabolic and central level, leading to a worsened version of AD pathology and memory dysfunction in the long term. Therefore, it remains possible that tight control of T2D could slow central pathology progression in AD. Acknowledgements: MG-A: Instituto de Salud Carlos III and FEDER (European Union), co-financed by Fondo Europeo de Desarrollo Regional "Una manera de hacer Europa" (PI12/00675), Fundacion Eugenio Rodriguez Pascual 2015, Junta de Andalucia, Consejeria de Economia, Innovacion, Ciencia y Empleo, Proyectos de Excelencia (P11-CTS-7847).

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Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

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Program#/Poster#: 40.09/C14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIA F30AG048710

NIH NINDS R01NS085171

Title: ΔFosB and epigenetic gene regulation in Alzheimer's disease

Authors: *J. YOU[1], M. PYFER[1], I. PETROF[1], K. MURALIDHARAN[1], C.-H. FU[1], U. TOSI[1], B. CORBETT[1], X. ZHANG[1], E. NESTLER[2], J. CHIN[1];
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Abstract: Alzheimer's disease (AD) is associated with an increased incidence of seizures, and recent evidence suggests that such neuronal hyperexcitability in AD may actively contribute to cognitive decline. Therefore, understanding the mechanisms by which seizures contribute to cognitive decline may enable the discovery of novel therapeutic targets. Our studies of seizure-related changes in the hippocampus of a transgenic mouse model of AD have revealed that expression of the transcription factor ΔFosB is markedly increased by seizures. Unlike other leucine zipper transcription factors, ΔFosB has an unusually long half-life, and this unique property may explain how even a few intermittent seizures can cause long-lasting changes in gene expression that affects cognition. Our studies demonstrate that viral overexpression of ΔFosB in the hippocampus of wildtype mice is sufficient to alter hippocampal gene expression and cause cognitive deficits similar to those we observe in AD transgenic mice. Therefore, further delineation of ΔFosB's functions and identification of its targets in the hippocampus may provide critical insight into the mechanisms by which seizures induce synaptic dysfunction and cognitive impairment in neurological conditions such as epilepsy and AD.

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Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5P01-AG05119

NIH Grant P30-AG028383

Title: Quantification of cytosine modifications in Alzheimer's disease

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Abstract: DNA methylation of the fifth position of cytosine is a key epigenetic modification regulating gene transcription. Although epigenetic modifications to cytosine have been extensively studied in embryonic development and cancer, there has been little study of epigenetics as it relates to neurodegeneration, particularly Alzheimer's disease (AD). Studies of global levels of 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC), as well as gene modifications in target brain regions, could provide insight regarding neurodegeneration and dysfunction as it relates to AD. Bulk level changes to modified cytosine could suggest changes in epigenetic marks on the genetic level, giving insight to possible altered transcriptional states. Analysis of global cytosine modifications, as well as genetic levels of cytosine, 5-mC, and 5-hmC, would give a more comprehensive picture of epigenetic modification as it relates to AD. To determine global modified cytosine profiles in the brain of AD and age-matched normal control (NC) subjects, a GC/MS method was developed using stable labeled standards of cytosine, 5-mC, and 5-hmC. Bulk levels of epigenetic marks were quantified in key brain regions of AD and NC subjects, expressed as percent of control values. Preliminary data show elevated levels of 5-mC in AD subjects compared to NC in disease associated brain regions. To determine if 5-mC oxidation is altered in target genes associated with AD, specific restriction endonuclease cleavage at target CCGG sequences, paired with quantitative polymerase chain reaction (q-PCR), was used to quantify sequence specific levels of cytosine, 5-mC, and 5-hmC in a subset of AD relevant genes. DNA samples were isolated from the hippocampus/parahippocampal gyrus (HPG) of AD and NC subjects. To correlate epigenetic modification to transcriptional regulation, changes in protein levels were determined using Western blot analysis. Our data suggest significantly altered levels of epigenetic marks in genes relevant to AD in the HPG of AD subjects compared to NC subjects. Collectively, these studies suggest epigenetic modifications to cytosine may be associated with AD and potentially play a role in neurodegeneration and dysfunction.

Disclosures: E.M. Ellison: None. **M.A. Bradley-Whitman:** None. **M.A. Lovell:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.11/C16

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Clusters of damaged neuronal terminals (dystrophic neurites) form around ruptured capillaries in Alzheimer's disease

Authors: *G. K. HANSRA, K. M. CULLEN, G. POPOV, P. O. BANCZEK;
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Abstract: Rationale: Growing evidence suggests that vascular pathology may be important in the pathogenesis Alzheimer's disease (AD). Cerebrovascular abnormalities are common with increasing age and have been associated with cognitive impairment in AD. Previously, we showed that all amyloid-containing plaques are found at sites of capillary damage. These plaques colocalise with haemorrhage markers and inflammatory cells. We now have substantial evidence that senile plaques are, in fact, microhaemorrhages of 100-200μm. Linking the neuritic degeneration to these microstrokes would provide a unifying hypothesis for the vascular aetiology of the hallmark AD lesions. **Objective:** To correlate the location of neuritic plaques (clusters of hyperphosphorylated tau dystrophic neurites) to the microvascular network in *post mortem* brain tissue from AD and neurologically normal patients. **Methods and Results:** Blocks of formalin fixed human brain tissue were obtained from the Australian Brain Bank Network. This study was approved by the Human Research Ethics Committee (HREC), University of Sydney. Cases were chosen with a range of neuritic pathology using the Braak and Braak stageing criteria ranging from stages 0 to VI. Cases included 5 neurologically-normal individuals. Blocks of hippocampus, superior frontal and cingulate cortices were cryosectioned at 50-200μm and fluorescently labeled for tau (AT8) and collagen IV (for blood vessels). Entire neuritic plaques contained within the depth of the section were optically sectioned. Each plaque was then examined for the presence of a microvessel. Imaris and Fiji applications were used to measure features of the microvessels including length density, tortuosity, diameter, branch density as well as, distance to lesions. We find that neuritic plaques encircle capillaries in all stages of disease. We also find that neuritic plaques frequently appear around collapsed vessels and near capillary branches. Moreover, dystrophic neurites within the microhaemorrhages are iron-positive, suggesting iron uptake from the haemorrhage site. We also note iron-positive neurites emanating from the microstroke region. We suggest that this iron is derived from the leakage of blood into the parenchyma. **Conclusion:** This study provides evidence that the clinically-significant neuritic lesions of AD form at sites of microhaemorrhage, which strengthens the notion, that vascular breakdown is the proximal cause of AD. Therapeutic and preventative strategies aimed at vascular health have potential to delay or slow the progression of this disease.

Disclosures: **G.K. Hansra:** None. **K.M. Cullen:** None. **G. Popov:** None. **P.O. Banczek:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG044712

Saint Mary's Foundation

Title: Differential gene expression profiles in vascular dementia and Alzheimer's disease

Authors: ***E. MCKAY**[1,2], J. S. BECK[1], M. E. WINN[4], K. DYKEMA[4], A. P. LIEBERMAN[5], H. L. PAULSON[6], S. E. COUNTS[1,2,3,7];
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Abstract: Longer life expectancies have contributed to an increased prevalence of dementias in the aged population. The two most prevalent causes of dementia are Alzheimer's disease (AD) and vascular dementia (VaD). However, the pathogenic mechanisms that differentiate these two diseases are unclear. To begin to understand these differences, we used Agilent Human 8x60K v2 microarrays to profile gene expression in frozen frontal cortex tissue samples (BA 10) harvested postmortem from individuals who died with probable AD, VaD (multi-infarct subtype), or no dementia (n = 11-12/group). The cases were matched for age, postmortem interval, and gender. Of the 24,127 genes featured on the microarray platform, 3495 genes were uniquely dysregulated in AD (1344 up; 2151 down). Notably, preliminary network and pathway analyses revealed that the genes specifically downregulated in AD but not in VaD were heavily enriched for synaptic function (e.g., synapsin I, rab3a), axonal transport (e.g., dynein, bassoon), and glutamatergic signaling (e.g., NR2, mGluR5). Hence, the molecular pathogenic mechanisms leading to VaD may be different from AD. In this regard, microarray analysis revealed that 413 genes were uniquely dysregulated in VaD (221 up; 192 down). Preliminary network analysis revealed a prominent hub centered on the small GTPase Rac1, a critical negative regulator of epithelial antioxidant and neurotrophic activities. Rac1 was significantly down-regulated in VaD

compared to AD and controls (log fold change = -0.4, FDR adjusted $p < 0.01$). Likewise, several Rac1 inhibitors (e.g., RalBP1) were upregulated whereas Rac1 activators (e.g., puratrophin-1) were downregulated in VaD. Taken together, these results suggest a compensatory pathway is activated in VaD that inhibits Rac1-mediated oxidative stress and pro-apoptotic function. This pathway may be amenable to therapy for early intervention in VaD. In addition, we found that the histone acetyltransferase CREB-binding protein (CREBBP) was significantly upregulated in VaD samples (log fold change = 0.4, FDR adjusted $p < 0.02$). Network analysis revealed CREBBP to be a modulatory hub for several upregulated genes encoding core histones (e.g., H2, H4) and regulators of chromatin remodeling (e.g., SATB1), indicating that distinct epigenetic programs are activated in VaD compared to AD. These findings provide insight into the differential pathogenic mechanisms underlying the two most prevalent dementias. These differences could prove vital for driving dementia-specific treatments and prevention strategies.

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Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.13/C18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 NS051874

Belfer Neurodegeneration Consortium

Title: Generation of isogenic iPS cells to address the effects p25/Cdk5 activity on AD pathology

Authors: *J. SEO, Y.-T. LIN, R. MADABHUSHI, L.-H. TSAI;
Picower Inst. for Learning and Memory, Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Aberrant activity of cyclin-dependent kinase 5 (Cdk5) has been observed in Alzheimer’s disease (AD), and this is associated with increased p25, the proteolytic fragment of p35. Previously, we showed that the blockade of p25 generation attenuates AD pathological phenotypes including gliosis and synaptic dysfunction in 5XFAD mice. However, because of the lack of tauopathy phenotypes in most of AD mouse models harboring mutations in amyloid beta precursor protein (APP) or presenilin 1 (PSEN1) locus, and the biochemical and physiological differences between mouse models and humans, further investigation on the effects of p25 on

tauopathy and the beneficial effects of p25 inhibition on AD pathology in human systems are required. To address this, we take advantage of induced pluripotent stem cells (iPSCs) derived from fibroblasts of healthy donors or AD patients with the pathogenic M146I mutation or the deletion of exon 9 in PSEN1 locus. We first differentiated iPSCs to neural progenitor cells (NPCs), and observed more DNA damages and increased levels of histone deacetylase 2 (HDAC2) in NPCs harboring PSEN1 mutations compared to controls. To address whether inhibition of p25 generation is beneficial, we utilized the CRISPR/Cas9 genome editing tool to created isogenic lines in which the endogenous p35 is replaced with a cleavage-resistant p35. We also created isogeneic iPSC lines with PSEN1 mutations corrected. We are currently investigating neuronal differentiation, synaptic function and pathology exhibited by neurons derived from these multiple pairs of cells.

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Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Jeane B. Kempner Predoctoral Fellowship

Title: Phosphorylation of FGF14 at S226 in the tg2675 mouse model of Alzheimer’s disease is rescued by PPAR-gamma agonist RSG

Authors: *W.-C. HSU[1], E. WOLD[2], A. OCON[2], S. ALI[3], N. PANOVA[3], J. HAIDACHER[4], L. DENNER[4], K. T. DINELEY[5], F. LAEZZA[3];
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Abstract: Recent research has suggested that cognitive impairment associated with Alzheimer’s disease (AD) in humans, as well as Aβ pathology associated with animal models such as Tg2576 can be ameliorated with treatments that target the nuclear receptor peroxisome proliferator-activated receptor-gamma (PPAR-gamma), such as the PPAR-gamma agonist rosiglitazone (RSG). Previously, we have demonstrated that RSG treatment of the Tg2576 animal model normalizes hyperactivity of granule neurons in the dentate gyrus (DG), the brain area critical for memory formation, and rescues cognitive deficits. We now show through LC-MS/MS that phosphorylation of fibroblast growth factor 14 (FGF14), an accessory protein of the voltage-

gated Na+ complex (Nav) critical for neuronal firing, at S226 is reduced following treatment of Tg2576 mice with RSG. Using the split-luciferase assay to screen for FGF14:Nav1.6 complementation in recombinant cells treated with kinase inhibitors revealed that the effects of PKC and Wee1 inhibitors were abrogated by the phosphosilent mutation S226A. Pathway analysis suggests that PKC forms a PPAR-gamma-centered network of kinases sensitive to RSG. In total, these results suggest a novel role of FGF14 as a PPAR-gamma-sensitive target controlling Aβ-induced dysfunctions of neuronal activity of DG, leading to memory impairment in early AD.

Disclosures: **W. Hsu:** None. **E. Wold:** None. **A. Ocon:** None. **S. Ali:** None. **N. Panova:** None. **J. Haidacher:** None. **L. Denner:** None. **K.T. Dineley:** None. **F. Laezza:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.15/C20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Georgetown University Funding

Title: Ubiquitin specific protease (USP)-13 regulates Parkin function and modulates intracellular Aβ and extracellular plaque levels in AD models

Authors: ***M. HEBRON**, I. LONSKAYA, Y. FENG, M. IBA, C. MOUSSA;
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Abstract: Parkin is an E3 ubiquitin ligase that tags specific protein substrates with ubiquitin and targets them for degradation. Parkin is usually auto-inhibited and inactive, but self-ubiquitination leads to Parkin activation, which can be regulated by de-ubiquitinases (DUBs). De-ubiquitination de-activates Parkin and may reduce its recognition by the proteasome, leading to accumulation of insoluble aggregates reminiscent of PD and AD pathologies. Our new data show increased USP13 levels (2-fold) in post-mortem AD and PD brains, along with increased insoluble Parkin levels. Cell culture studies show that USP13 over-expression de-ubiquitinates Parkin, leading to soluble and insoluble protein accumulation and decreased proteasome activity. Conversely, USP13 knockdown via shRNA reverses these effects. Lentiviral USP13 was injected into the hippocampus of 4-6 months old transgenic APP mice, harboring Swedish, Iowa and Dutch mutations (TgAPP). USP13 expression altered soluble and insoluble Parkin levels, suggesting regulation of Parkin stability, and thereby activity. USP13 overexpression reduced intracellular

Aβ42 but led to more plaque deposition, while USP13 knockdown via shRNA decreased both intracellular Aβ42 and extracellular plaques, suggesting that USP13 plays a role in intracellular degradation of Aβ42. Consistently, USP13 overexpression increased p-Tau, but USP13 shRNA reduced p-Tau levels. Taken together these data suggest that an increase in USP13 leads to Parkin de-ubiquitination, affecting its stability to facilitate intracellular Aβ42 and p-Tau clearance. These studies suggest that USP13 may be a drug target that regulates Parkin activity to induce autophagic protein clearance in neurodegenerative diseases.

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Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

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Program#/Poster#: 40.16/C21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Harry Botterell Foundation

The Canadian Vascular Network

Title: Aldehyde dehydrogenase 2 null mice as an oxidative stress-based model of cognitive impairment and sporadic Alzheimer’s disease that displays both neuronal and vascular pathologies

Authors: A. ELHARRAM, Y. D'SOUZA, R. D. ANDREW, *B. BENNETT;
Biomed. and Mol. Sci. and Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada

Abstract: The study of late-onset/age-related Alzheimer’s disease (AD)(sporadic AD, >95% of AD cases) has been hampered by a paucity of animal models. Oxidative stress is considered a causative factor in late onset/age-related AD, and aldehyde dehydrogenase 2 (ALDH2) is important for the catabolism of toxic aldehydes associated with oxidative stress. One such toxic aldehyde, the lipid peroxidation product 4-hydroxynonenal (HNE), accumulates in the brains of AD patients and is associated with AD pathology. Given this linkage, we hypothesized that in mice lacking ALDH2, there would be increases in HNE and the appearance of AD-like pathological changes. Accordingly, changes in relevant AD markers in Aldh2 null mice and their wildtype littermates were assessed over a 1 year period. Marked increases in HNE protein adducts arise in hippocampi from Aldh2 null mice as early as 3 months, and there were age-

related increases in amyloid-beta (Aβ), phospho-tau protein, and activated caspases 3 and 6. Also observed were age-related decreases in PSD95 and synaptophysin (indicating synaptic loss), pGSK3β, and total and phosphorylated CREB. Aldh2 null mice exhibit a graded, age-related decrease in performance in the novel object recognition and Y maze tasks beginning at 3 months and maximal at 6-7 months. There was decreased performance in the Morris Water Maze task in 6 month old Aldh2 null mice. Differences in locomotor activity and coordination, as well as behavioural phenotype using the open field test, balance beam and SHIRPA standardised battery were not observed, suggesting that diminished cognitive performance was the result of impaired memory and not due to confounding changes in motor function. These mice also exhibit dystrophic neurites and loss of dendritic spines, brain atrophy, defects in cholinergic agonist-induced CREB and ERK phosphorylation, endothelial dysfunction, increased monomeric and oligomeric Aβ in cerebral microvessels, and cerebral vascular microbleeds. Additionally, chronic administration of the novel nitrate, 4-methyl-5-(2-nitroxyethyl) thiazole, a neuroprotectant that targets synaptic failure via activation of NO/cGMP/pCREB signaling, or of the HNE scavenger, histidine hydrazide, reverses the progressive memory deficits and biochemical changes that occur in these mice. The presence of AD-like pathologies and the reversal of memory and biochemical deficits by pharmacological intervention suggests that Aldh2 null mice represent a new model of age-related cognitive impairment and sporadic AD that may prove useful both for assessing AD therapeutics and for gaining better insight into the pathogenesis of AD.

Disclosures: **A. Elharram:** None. **Y. D'Souza:** None. **R.D. Andrew:** None. **B. Bennett:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.17/C22

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The effects of metabolic syndrome in the brain structures and its implications in the pathogenesis of neurodegenerative disease

Authors: *L. PEREIRA[1], J. J. MELOT[1], E. CASTRO[2], C. M. MORÁN[3];
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Abstract: Metabolic syndromes have been recently identified as a group of signs and symptoms that greatly increase the risk for many conditions later in life such as diabetes, cardiovascular diseases, and steatosis among others. Some of these metabolic changes, include insulin resistance

and high cholesterol, have been shown to cause defects in the neurochemistry and influence neurologic diseases such as Alzheimer's disease. Given that such metabolic disturbances might contribute to neurodegenerative disease, it opens up a novel field of study. Apolipoprotein E (ApoE) catabolizes lipids and cholesterol, and ε4 allele is associated to Alzheimer's disease. Objective: Identify brain structures directly affected by high fat diet (HFD)-induced metabolic syndrome and in genetically predisposed ApoE-/- mice. Methods: C57BL/6 wild-type and ApoE-/- mice were HFD (41% fat, 17% protein and 43% carbohydrate) fed for 12 weeks. All animals had a metabolic panel examination in order to quantify metabolic disturbances, and presented hyperlipidemia, hyperglycemia, glucose intolerance, and steatosis. Brains from mice were extracted. Two groups were examined: control (wild-type) and experimental (ApoE -/-). Sagittal sections of 8µm and H&E stained were prepared and neurologic comparisons were made between the groups. Results: In comparison with wildtype (Wt), ApoE-/- group had an increase number of xanthomas (lipid aggregates) within the cerebral cortex [Wt = 7 aggregates per field view (SD +/-2), ApoE-/- = 19 aggregates (SD +/-2]; and hippocampi [Wt = 10 aggregates per field view (SD +/-3), ApoE-/- = 37 aggregates (SD +/-2]. Increased number of immune cells concentrated within the brain blood vessels was observed. Remarkably, cerebellar changes were detected in ApoE-/- mice under HFD such as decreased number of Purkinje cells [Wt = 131 cells counted (SD +/-14), ApoE-/- = 62 cells (SD +/-2]; and reductions within the molecular layer length [Wt = 68.75 length (SD +/-4), ApoE-/- = 47.75 length (SD +/-6]. Conclusions: Lipid aggregations resemble the amyloid plaques found in Alzheimer's brains. These xanthomas may disrupt the neuronal signaling within the cerebrum. Linking metabolic alterations to the onset of neurodegenerative disorders, such as Alzheimer's disease, is an important scientific advancement. In the cerebellum, damage to Purkinje and molecular layers might lead to loss of body balance and posture, and degeneration of motor learning and gait. The overall outcomes of these studies will contribute to understand the impact of nutrition on the neuroanatomy and its potential consequences on cognition, motor functions and pathogenesis of neurological diseases.

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Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.18/C23

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: An insight on new roles of the proteasome complex on organelle axonal transport

Authors: *M. G. OTERO[1], T. M. M. SAEZ[2], M. ALLOATTI[1], L. E. CROMBERG[1], T. FALZONE[1,2];
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Abstract: Axonal transport is the neuron mechanism that ensures the correct delivery and positioning of different organelles and vesicles. Impairments of the transport machinery at different levels have been associated with abnormal protein accumulation in Alzheimer disease (AD). The observation in AD of abnormal protein accumulation at confined intracellular regions of the neuron suggests local defect in protein degradation. The ubiquitin-proteasome system (UPS) is the major route for cytosolic protein degradation and proteasome distribution throughout the neuron is necessary for normal physiological neuronal functions and is abnormal in disease. We have recently demonstrated using a novel high spatial- and temporal-resolution analysis that proteasomes have 3 different motion regimes in axons. We described the active transport of proteasomes that is dependent on motor function and association of membrane organelles. These novel and important results position the axonal transport of the proteasome as a relevant intracelluar mechanism that can be impaired during the progression of neurodegenerative diseases. Here we tested a pathway to improve the axonal transport of proteasomes by the overexpression of the adaptor proteasome protein ECM29 that has affinities for proteasome, motor and membranes. In addition, the role of proteasome activity in the regulation of the axonal transport dynamics of other axonal cargos was tested in condition of short proteasome inhibitions with MG132. ECM29 overexpression is capable to improve the anterograde segmental velocities of proteasomes. Moreover, Proteasome inhibition revealed a great impact on transport dynamics inducing specific reduction in proportions and velocities of movement for different cargos. Taken together, our results reinforce the relevance of our recent demonstration of the axonal transport regimes that ensure the homogenous distribution of proteasomes in neurons and suggest that proteasome activity impacts on the regulation of the axonal transport dynamics. Interestingly, we propose a mechanism to improve the distal distribution of proteasomes in synapses that can be a novel mechanism to recover the local synaptic defects in protein degradation that have been described in neurodegenerative diseases such as AD.

Disclosures: **M.G. Otero:** None. **T.M.M. Saez:** None. **M. Alloatti:** None. **L.E. Cromberg:** None. **T. Falzone:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.19/C24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FAPESP

CNPq

CAPES

USP-NAPNA

Title: Streptozotocin-induced neurodegeneration involves NADPH oxidase activation

Authors: *K. G. RAVELLI, B. A. ROSÁRIO, M. S. HERNANDES, L. R. G. BRITTO; Dept. of Physiol. and Biophysics of the Inst. of Biomed. Sci., Univ. of São Paulo, São Paulo, Brazil

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the progressive loss of memory, disordered cognitive function, decline of the language function, and behavioral changes, including paranoia, delusions and loss of social adequacy. The neuronal degeneration in the nervous system of patients with AD has been linked to oxidative damage to several types of biomolecules. Some studies have suggested the involvement of NADPH oxidase (Nox), a multisubunit enzyme that catalyzes the reduction of oxygen, in the physiopathology of neurodegenerative diseases. The main purpose of this study was to investigate the involvement of NADPH oxidase in memory, inflammation and neuronal death in the hippocampus in the streptozotocin(STZ)-induced AD mouse model by comparing the effects of that drug on mice lacking gp91phox-/-, the catalytic subunit of Nox2, and wild type mice (C57BL/6). The AD-like condition was induced by intracerebroventricular stereotaxic injections of streptozotocin in male mice (25-30g) (Committee for Ethics in Research no 098/2012). In control animals we injected citrate buffer. The animals were subjected to behavioral testing or were euthanized for analysis 14 days after the surgery. In addition to the behavioral analysis, using the object recognition test, immunoblotting techniques were used to determine the protein expression of astrocyte and microglia markers and caspase-3, and immunohistochemical methods were used to assess the activation of astrocytes and microglia in the CA1 and CA3 areas of the hippocampus. Wild-type animals injected with STZ showed a reduction of 26% and 29% in the short and long term memories, respectively. Furthermore, they showed increased expression of astrocyte markers (58%), microglial markers (76%) and caspase-3 (690%). The increased expression of astrocyte and microglia markers occurred in both hippocampal regions studied. The knockout mice treated with STZ showed no change in any of those analyses, when compared to the respective control group. These data suggest that NOX2 - mediated oxidative stress contributes to neurodegeneration that occurs in AD, since the knockout animals showed a resistance to STZ-induced processes in the nervous system.

Disclosures: **K.G. Ravelli:** None. **B.A. Rosário:** None. **M.S. Hernandes:** None. **L.R.G. Britto:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.20/C25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: TWU Department of Biology

TWU Research Enhancement Program

Title: Small Rho GTPases affect production and localization of Alzheimer's disease proteins: APP, Aβ, and Tau

Authors: ***R. CHABAYTA**, P. MODY, J. REDDY, D. L. HYNDS;
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Abstract: Alzheimer's disease (AD) is a fatal neurodegenerative disorder that is histopathologically characterized by the formation of amyloid plaques and neurofibrillary tangles in the brain. Aberrant cleavage of the amyloid precursor protein (APP), a transmembrane protein, produces beta amyloid plaques. hyperphosphorylation of a microtubule associated protein, tau, forms the neurofibrillary tangles. Defects in the Rho family GTPases (small guanosine triphosphatases) signaling pathways have been implicated in many neurological diseases, including Alzheimer Disease. Rho proteins (Rho, Rac, Cdc42) regulate a wide variety of cellular functions and play key roles in actin cytoskeletal rearrangements. In this study, we determined the effects of manipulating activity of the Rho GTPases, Rho, Rac, Cdc42, on the production of Alzheimer's disease proteins. For that purpose, B-35 cells were treated with the Rho GTPase inhibitors and activators. The levels of APP, Aβ, and tau were determined by western blotting and immunocytochemistry. Toxin A, a Rho/Rac/Cdc42 inhibitor, increased the levels of Aβ and tau as expected and decreased the levels of APP. Rho inhibitor I and Rho/Rac/Cdc42 activator treatments significantly decreased APP levels, but increased the high and low molecular weight levels of tau. Rac I Inhibitor II decreased the levels of APP, suggesting that disruption or manipulation of Rho GTPases affect APP, Aβ, and tau production. To further investigate the role of Rac in AD pathology, cytosolic and membrane lysates from cells transfected with EmGFP (without Rac1), EmGFP-Rac1 and EmGFP-Rac1^{C196A} were used to detect changes in APP and tau levels in the cytosol and at the membrane. High molecular weight of APP increased and tau

levels decreased in the cytosol when transfected with EmGFP-Rac1 and EmGFP-Rac1C196A. While low molecular weight of APP decreased in the cytosol only when transfected with EmGFP-Rac1. Our data support a role for Rho GTPases in the classic hallmarks of AD pathology and suggest targeting Rac as a possible AD therapy.

Disclosures: **R. Chabayta:** None. **P. Mody:** None. **J. Reddy:** None. **D.L. Hynds:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.21/C26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RSG Grant

ILDPH Grant

Title: Kir6.2 is increased in astrocytes of the 3xtg mouse hippocampus

Authors: ***C. M. GRIFFITH**[1,3], A. A. SHARP[1,2,3], X. X. YAN[4], P. R. PATRYLO[1,2,3]; [1]Physiol., [2]Anat., Southern Illinois Univ. Sch. of Med., Carbondale, IL; [3]Ctr. for Integrated Res. in Cognitive and Neural Sci., Southern Illinois Univ., Carbondale, IL; [4]Anat. and Neurobio., Central South Univ. of Xiangya Sch. of Med., Changsha, China

Abstract: KATP channels are inwardly rectifying potassium channels composed of 4 pore-forming subunits (Kir6.1 or Kir6.2) and 4 sulphonylurea regulatory subunits (SUR1, SUR2A, or SUR2B). These channels couple neuronal activity with metabolism. One of the hallmarks of Alzheimer’s disease (AD) is brain hypometabolism and recent data (Liu et al. JAD, 2010) show that treating the 3xTg mouse model of AD with diazoxide (a KATP channel agonist) improves memory and reduces β-amyloid plaques and neurofibrillary tangles. Whether AD is associated with a change in KATP channels is unknown. To test this hypothesis we examined Kir6.1 and Kir6.2 in the hippocampi of aged male 3xTg and C57/129 control mice using western blots and immunohistochemistry. For immunoblots, the plasma membrane fraction was separated from total membrane using differential centrifugation. Protein concentrations were determined using the Bradford assay and 50µg were loaded on 10% SDS-Page gels, transferred to PVDF membranes and probed for Kir6.1 or Kir6.2. Appropriate secondary antibodies were used for densitometric quantification of protein bands using the Odyssey Licor system. This revealed no change in total or plasma membrane Kir6.1 but a significant increase in plasma membrane Kir6.2

(wild type NOD = 1 ± 0.06 n = 12, 3xTg NOD = 1.60 ± 0.24, n = 11; $p < 0.05$ student's t-test). To then assess regional and cell specific differences in Kir6.2, immunohistochemistry was used. Mice were perfused intracardially with 4% paraformaldehyde, brains were extracted, post-fixed overnight and cryoprotected in 30% sucrose. 30µm coronal sections were then processed for DAB staining or immunofluorescence. DAB staining was done via normal procedures. Immunoreactive product (IR) was visualized using 0.003% H2O2 and 0.05% DAB. This revealed an increase in Kir6.2 IR in "presumptive astrocytes" in 3xTg mice. To verify the localization of Kir6.2 in astrocytes and semi-quantify astrocytic and neuronal Kir6.2 IR triple immunofluorescence was used. Sections were blocked for 1 hour in 5% rabbit serum in PBS and then incubated in primary antibody overnight with an appropriate secondary used for Kir6.2 visualization. Immunofluorescence confirmed that Kir6.2 was present in GFAP-IR astrocytes. While the physiological consequences of an increase in Kir6.2 expression in reactive astrocytes is unclear, it is possible that this change could affect K+ and glutamate buffering. Future electrophysiology experiments will be used to assess these possibilities.

Disclosures: **C.M. Griffith:** None. **A.A. Sharp:** None. **X.X. Yan:** None. **P.R. Patrylo:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.22/C27

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: grant GRS-002/13

Title: Reduction of Alzheimer's disease beta-amyloid pathology in the absence of gut microbiota

Authors: *T. HARACH;
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Abstract: Alzheimer's disease (AD) is the leading cause of dementia in western societies that features tremendous societal and personal cost and burden. To date, there is no early diagnosis or cure for this devastating neurodegenerative disorder. Gastro-intestinal microbiota is an essential factor to many physiological processes including nutrition, inflammation, vitamins synthesis, drug processing, but also defense against pathogens. A growing body of evidence suggests that gastro-intestinal microbiota impacts on brain disorders such as autism and Parkinson's disease. However, the role of the intestinal microbiota in AD has never been investigated. Our preclinical results using axenic mouse models of AD indicate that gastro-intestinal microbiota is involved in

the development of AD and supports the view that neurodegenerative disorders may be tackled by gastro-intestinal modulation.

Disclosures: T. Harach: None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.23/C28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MALAT Family, Technion

Title: Decline in central cholinergic activity alters central and peripheral immune response

Authors: *B. KORIN[1], T. BEN-SHAANAN[1], H. AZULAY-DEBBY[1], F. HAKIM[2], A. ROLLS[1];
[1]Immunol., Technion, Haifa, Israel; [2]Pediatric Pulmonary Unit, Rambam Hlth. Care Campus, Haifa, Israel

Abstract: The tight link between Alzheimer's disease (AD) and aging suggests that processes occurring in the aging brain promote AD initiation and progression. Understanding the transition from normal aging to AD pathology is crucial for early detection, prevention and treatment of the disease. Studies suggest that the immune system is involved in this process, as activated immune cells, including microglia/macrophages, are evident in diseased brains. Others argue that these immune cells act to restore tissue homeostasis, activated in response to the age-related changes in the neural tissue. Among those changes is the decrease in cholinergic activity and shrinkage of cholinergic neurons in the basal forebrain (BF). Previous lesion-based studies linked the activity of BF cholinergic neurons with immune changes, however, this research direction is very limited. We utilized the newly developed engineered GPCRs (DREADDs), to specifically attenuate the activity of these neurons in mice, simulating the decline in their activity with age. Subsequently, we characterized the effects of this neuronal manipulation on the immune system. Using high-throughput analysis of the immune system, we discovered that inhibition of BF cholinergic neurons activity resulted in significant changes in monocytes/macrophages phenotype and phagocytic activity in the periphery and changes in monocytes/microglia activity in the brain. Taken together, we establish a new experimental paradigm to study the effects of BF cholinergic neurons activity on aging and AD, and demonstrate that changes in the cholinergic activity of the brain can affect the peripheral and central immune response.

Disclosures: **B. Korin:** None. **T. Ben-Shaanan:** None. **H. Azulay-Debby:** None. **F. Hakim:** None. **A. Rolls:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

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Program#/Poster#: 40.24/C29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Owens Family Foundation

NIH

University of Virginia President's Fund for Excellence

Webb and Tate Wilson

Fraternal Order of Eagles

Title: Neuronal cell cycle re-entry and mTOR: how insulin resistance promotes Alzheimer's disease

Authors: A. NORAMBUENA[1], H. WALLRABE[1], L. MCMAHON[1], E. SWANSON[1], S. THOMAS[1], D. BAERTHLEIN[1], A. SILVA[1], E. KODIS[1], J. W. MANDELL[1], S. ODDO[2,3], *G. S. BLOOM[1];
[1]Univ. of Virginia, Charlottesville, VA; [2]Banner Sun Hlth. Res. Inst., Sun City, AZ; [3]Univ. of Arizona Col. of Med., Phienix, AZ

Abstract: Brain insulin resistance is a characteristic feature of Alzheimer's disease (AD), but how it leads to specific AD phenotypes has not been determined. Mechanistic target of rapamycin (mTOR), a protein kinase subunit of the mTORC1 and mTORC2 multiprotein complexes, plays a major role in cell proliferation, and is hyperactivated in AD. Insoluble aggregates of amyloid-β (Aβ) and tau respectively accumulate as plaques and tangles in AD brain, where ectopic neuronal cell cycle re-entry (CCR) leads to massive neuron death, which along with synaptic dysfunction, underlies memory and cognitive loss. We previously showed that CCR entails soluble Aβ oligomers (AβOs) activating fyn, PKA and CaMKII, which respectively phosphorylate soluble tau at Y18, S409 and S416. Now we report that Rac1-mediated mTOR dysregulation is also essential for AβO-induced CCR, which furthermore is insulin-sensitive. Inhibition or reduction of mTORC1, mTORC2 or Rac1 in cultured neurons

blocks AβO-induced CCR, which requires mTORC1-dependent tau phosphorylation at S262. In human brain, plaques and tangles strongly correlate with CCR, and with tau phosphorylation at S262, S409 and S416. CCR can be prevented in cultured neurons by reducing Rac1-dependent targeting of mTOR to the plasma membrane, forcing mTORC1 onto lysosomes, knocking down the lysosomal mTORC1 inhibitors, Nprl3 or Tsc2, or by insulin, which activates mTORC1 at lysosomes to inhibit autophagy. In AD model mice, genetic reduction of mTOR suppresses CCR and tau phosphorylation at S262, the latter of which is also reduced by rapamycin. Collectively, the data presented here establish mTORC1 activation at the plasma membrane, but not at lysosomes, as essential for AβO-induced, tau-dependent CCR. Because AβOs also trigger insulin resistance in AD, the new data suggest that AβOs dysregulate mTOR signaling by promoting mTORC1 activation at the PM while preventing insulin from stimulating mTORC1 at lysosomes. These dual effects of AβOs provide a mechanistic explanation at the cell biological level for classifying AD as type 3, or brain-specific, diabetes.

Disclosures: **A. Norambuena:** None. **H. Wallrabe:** None. **L. McMahon:** None. **E. Swanson:** None. **S. Thomas:** None. **D. Baerthlein:** None. **A. Silva:** None. **E. Kodis:** None. **J.W. Mandell:** None. **S. Oddo:** None. **G.S. Bloom:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.25/C30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health, USA

Title: 17 KDa and 23 KDa FGF-2 affect astrocyte proliferation and survival differentially

Authors: *Y. CHENG[1], Z. LI[2], P. Y. LOH[2];
[1]Developmental Neurobio., [2]NIH/NICHD, Bethesda, MD

Abstract: Astrocytes are the most abundant type of glial cells in the brain, and they play a key role in Alzheimer’s disease (AD). Amyloid beta (Aβ) toxicity is thought to be the cause of neuronal death and astrocyte dysfunction in AD brain. In the present study, we investigated the protective effects of low molecular weight (17 KDa) and high molecular weight (23 KDa) Fibroblast Growth Factor-2 (FGF-2) on Aβ-induced toxicity, and also the effects of FGF2 on astrocyte proliferation. Our results demonstrated that both isoforms of purified recombinant

FGF-2 had similar protective effects against Aβ1-42 induced toxicity in hippocampal neurons and cortical astrocytes as measured by LDH release assay. We then showed that the low molecular weight FGF-2 significantly promoted astrocyte proliferation as measured by Trypan Blue and DRAQ5 staining. In contrast, the high molecular weight FGF-2 did not have a significant effect on astrocyte proliferation. Furthermore, results from western blot suggested that both forms of FGF-2 activated ERK and AKT in a similar way within 30 min in astrocytes. Using specific inhibitors, we found that AKT signaling pathway but not ERK signaling pathway was required for the protective effects of FGF-2. Taken together, our findings revealed differential effects of 23 kDa and 17 kDa isoforms of FGF-2 on neuron/glial cell protection against Aβ toxicity and astrocyte proliferation, thus providing important information in tailoring the forms of FGF-2 to use as a potential therapeutic agent for treatment of AD.

Disclosures: **Y. Cheng:** None. **Z. Li:** None. **P.Y. Loh:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.26/C31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FSU College of Medicine

Title: Sex biases in susceptibility and severity of neurological disorders

Authors: *R. S. NOWAKOWSKI, J. L. BUNDY, C. M. VIED;
Biomed. Sci., FSU Col. of Med., Tallahassee, FL

Abstract: Sex differences in the brain are critical to understanding multiple neurological and behavioral disorders that differentially affect males and females, such as autism and Schizophrenia (more common in males), Alzheimer’s Disease and depression (more common in females). We generated transcriptomic data from the mouse hippocampus of six inbred strains of mice (129S1/SvImJ, A/J, C57BL/6J, DBA/1J, DBA/2J and PWD/Ph) that provide a novel and deep perspective on the issue of differences between males and females. Our data show that: 1) gene expression in male versus female mouse brains varies significantly, 2) there are 12 core genes that are differentially expressed across a diverse set of inbred strains, and 3) there are >2,500 non-core differentially expressed genes (DEGs) that vary from one inbred strain to another. To examine the diversity between the six strains, we categorized the DEGs in each strain according to sex-biased expression (a gene that is more highly expressed in males or

females). This analysis shows that DBA/2J is unique in having a majority of DEGs that are higher in females than in males, with 89% of all DEGs being female-biased. 129 is strongly male-biased, 69% of DEGs are more highly expressed in males. C57BL/6J and A/J are slightly male-biased. This diversity in sex-biased expression indicates that different genetic controls exist that regulate sex differences. This is significant and exciting because the existence of the non-core DEGs provides a basis for a mechanism to explain sex biases in disease susceptibility and severity. To gain insight into the function of the sex-biased DEGs, we have examined gene ontology (GO), pathway and phenotype enrichment. When analyzing all of the non-core DEGs from the six strains we found significant enrichment in phenotypes related to abnormal nervous system morphology and physiology, among others. In addition, several pathways were enriched significantly in the list of DEGs. Of particular interest was Alzheimer's disease (AD) enrichment, with 32 genes implicated in AD. Separate analysis of the male-biased and female-biased DEGs showed that 24 of the genes related to AD were female-biased and 8 were male-biased. Three of the male-biased genes have been implicated in a neuroprotective role in AD. These data suggest a genetic basis for the female-bias in AD that is independent of female longevity. To further elucidate the sex bias in AD we have, in progress, a transcriptomic analysis in an AD mouse model (5XFAD; Familial Alzheimer's Disease). Taken together, our transcriptomic data provides new insight into understanding the possible genetic bases for sex-specific susceptibility and severity of brain disorders.

Disclosures: R.S. Nowakowski: None. **J.L. Bundy:** None. **C.M. Vied:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P01 AG022550

NIH Grant P01 AG 027956

NIGMS P20 GM109098

Title: miR-146a dysregulates mitochondrial function and glycolysis

Authors: *S. JUN, S. N. SARKAR, J. W. SIMPKINS;
Physiol. and Pharmacol., West Virginia Univ. Hlth. Sci. Ctr., Morgantown, WV

Abstract: Mitochondrial dysfunction is associated with the aging process and with the pathogenesis of a variety of disorders such as metabolic syndrome and neurodegenerative diseases including Alzheimer's disease. MicroRNAs (miRNAs) are endogenous small RNAs of 21-25 nucleotides that post-transcriptionally regulate various gene expressions. Interestingly, miRNAs can modulate mitochondrial activity, and a discrete miR set has recently been identified in mitochondria of different species and cell types (mitomiRs) . Also, Ingenuity Pathway Analysis (IPA) of aging-related mitomiR targets has disclosed a number of resident mitochondrial proteins playing large roles in energy metabolism, mitochondrial transport and apoptosis. Among them, miR-146a, miR-34a, and miR-181a are involved in important cell functions (growth, proliferation, death, survival, maintenance) and age-related diseases. Furthermore, miR-146a was found to be significantly up-regulated in the temporal cortices of patients with AD. It was shown to function in the AD inflammatory and oxidative stress pathways, as it was shown to be up-regulated by NF-κB in response to Aβ42 in cultured human neuronal glial cells. However, the impact of miR-146a on mitochondrial function and glucose metabolism has not been analyzed. Since a single microRNA (miRNA) is capable of deregulating many genes' function, we hypothesized that increased miR-146a by Aβ may influence mitochondrial function and glucose metabolism, especially glycolysis through regulating multiple target proteins involved in these process in AD. We found that the level of miR-146a was significantly associated with Braak stage not only in temporal cortex but also in frontal cortex and cerebellum of AD patients. Through bioinformatic analysis study, we identified 4 putative target genes related to mitochondrial electron transport chain as well as 2 glycolysis related genes. Furthermore, overexpression of miR-146a significantly decreased ATP production, spare capacity, and maximum respiration measured in rat primary glial cell, and also significantly decreased glical glycolysis and glycolytic reserve that was measured by Seahorse assay. Our results indicate that increased level of miR-146a in brain cells may play a direct role in controlling mitochondrial function and energy metabolism by regulating mitochondrial protein expression as well as proteins regulating glycolysis. The modulation of miR-146a could thus mediate the loss of mitochondrial integrity and function in aging cells, inducing or contributing to the inflammatory response and to age-related diseases especially in AD.

Disclosures: S. Jun: None. **S.N. Sarkar:** None. **J.W. Simpkins:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.28/C33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIGMS 1SC3GM086323

CTSC #UL1-RR024996

G12 RR003037

G12 MD0075998

Title: The relationship between proteasome activity and changes in cellular levels of atp in a *Drosophila* model of Alzheimer's disease

Authors: *T. SCHMIDT-GLENEWINKEL[1,2], M. JANSEN[1], A. RASHID[1], J. NETHERCOTT[1], I. ONYEJIUKWA[1], S. BENNETT[1], A. KLEIN[1];
[1]Biol. Sci., Hunter Col. of CUNY, New York, NY; [2]Ph.D. Program in Biol., Grad. Ctr. of CUNY, New York, NY

Abstract: Many neurodegenerative diseases are associated with the formation of protein aggregates. The accumulation of aggregated proteins in form of neurofibrillary tangles and senile plaques in Alzheimer disease (AD) suggests that inability to turnover or degrade certain proteins might be a key factor in the etiology of certain neurodegenerative disorders, like Alzheimer disease. Degradation of most proteins is normally carried out via ubiquitination followed by degradation by the proteasome. Impairment of the ubiquitin proteasome pathway (UPP) may therefore be a contributing factor in the etiology of neurodegeneration. Several lines of evidence suggest that declining ATP levels as a consequence of deteriorating mitochondrial function may be relevant to the pathology of neurodegenerative diseases. To investigate the role between energy metabolism and proteasome function we will use the inducible GeneSwitch system to inhibit specific enzymes in energy metabolism via RNAi. Using the GeneSwitch system we also explore the relationship between impaired ATP synthesis and protein aggregation in a fly model of Alzheimer Disease. This approach allows us to study the interaction between reduced energy metabolism, reduced proteasome activity, and protein aggregation.

Disclosures: **T. Schmidt-Glenewinkel:** None. **M. Jansen:** None. **A. Rashid:** None. **J. Nethercott:** None. **I. Onyejiukwa:** None. **S. Bennett:** None. **A. Klein:** None.

Poster

040. Alzheimer's Disease: Beyond Abeta and Tau

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 40.29/C34

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant AG032297

Title: Preliminary investigation on the antidepressive effect of chronic oxotremorine treatment in a rodent model of Alzheimer's disease

Authors: *D. V. NAIR, M. M. AL-BADRI, H. PENG, J. PACHECO-QUINTO, C. B. ECKMAN, D. IACONO, E. A. ECKMAN;
Atlantic Hlth. Systems, Morristown, NJ and Biomed. Res. Inst. of New, Cedar Knolls, NJ

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease and the rate of progression varies from individual to individual. A great deal of evidence supports the idea that depression and other neuropsychiatric conditions co-exist with cognitive decline. However, the neurobiological basis of these symptoms and their influence on the clinical course of AD remain unclear. Our lab has shown previously that the 192-IgG saporin rat model of AD-like basal forebrain cholinergic cell loss exhibits a depression-like phenotype that develops months after the well-described impairment in spatial working memory. Furthermore, we have shown that chronic intracerebroventricular administration of the muscarinic agonist oxotremorine reverses both spatial working memory deficits and the depression-like behavior triggered by cholinergic denervation, and induces hippocampal neurogenesis. Current experiments are focused on determining additional pathological correlates of depression in this model and how they may be modulated by muscarinic agonists. To induce AD-like basal forebrain cholinergic cell loss, adult female Sprague Dawley rats were injected intracerebroventricularly (icv) with the immunotoxin 192-IgG-saporin (SAP) or saline as control (SHAM). After a 5 week recovery period, the rats received either 2 or 6 weeks of icv infusion of either oxotremorine or vehicle (saline) via osmotic minipump. Behavioral testing to assess the depressive phenotype was carried out using the sucrose consumption test every 2 weeks during oxotremorine treatment. The phenotype was further confirmed by forced swim test. The levels of ChAT, tryptophan hydroxylase (TPH), muscarinic receptors and FosB and ΔFosB were assessed in the hippocampus, basal forebrain, and orbitofrontal cortex by western blot and immunohistochemistry. Our preliminary results show increases in TPH, M1 receptors and FosB in the hippocampus, basal forebrain, and orbitofrontal cortex of a subset of treated animals, but no changes ChAT or ΔFosB. Further experiments are in progress to determine if there are changes in the expression of these and additional proteins in other brain regions including the nucleus accumbens, an area involved in activational aspects of motivation which also contributes to behavioral disorders such as to depression. The results of these studies may provide new insight in understanding the molecular basis of depression and antidepressant action of oxotremorine thereby defining new targets for possible therapeutic intervention for depressive symptoms in AD.

Disclosures: **D.V. Nair:** None. **M.M. Al-Badri:** None. **H. Peng:** None. **J. Pacheco-Quinto:** None. **C.B. Eckman:** None. **D. Iacono:** None. **E.A. Eckman:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

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McKeegan Graduate Fellowship

Purdue Research Foundation Fellowship

Floss Endowment Research Award

Title: Impaired alpha synuclein membrane interaction promotes aggregation and neurotoxicity in Parkinson's disease

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Abstract: Oligomerization of the protein alpha-synuclein (aSyn) is a critical factor in the onset of both genetic and sporadic Parkinson's disease (PD). In healthy individuals aSyn exists in a number of conformations, including a membrane-bound state which displays resistance to aggregation. How this aggregation-resistant state is altered is significant in PD research as a better understanding of the events that initiate aSyn aggregation is critical for designing neuroprotective strategies. We hypothesize that disruption of interactions between aSyn and phospholipid membranes leads to a shift to a conformation which is more susceptible to the formation of neurotoxic aSyn oligomers due to exposure of the central hydrophobic NAC region. Therefore, stabilization of membrane bound aSyn should prevent membrane-induced aggregation and alleviate aSyn neurotoxicity. We generated aSyn variants predicted to disrupt aSyn-membrane interactions including the genetic mutants A30P and G51D and a designed variant A29E. We characterized the variants' association with synthetic phospholipid vesicles and their ability to undergo membrane-induced aggregation. Dopaminergic neurotoxicity was examined using adenoviral-mediated expression in a primary midbrain culture model system. To examine

the potential protective effects of stabilization of aSyn-membrane interactions, we investigated the impact of endosulphine alpha (ENSA), a protein found to interact with membrane-bound aSyn, on membrane-induced aSyn aggregation and aSyn neurotoxicity. We found that the familial mutants A30P and G51D displayed increased exposure of the NAC region at the membrane surface, and this effect correlated with enhanced accumulation of potentially toxic, SDS-resistant aggregates at the membrane. Although A29E adopted a similar exposed conformation, this variant had a reduced propensity to undergo membrane-induced aggregation and also did not elicit neurotoxicity. Upon co-incubating aSyn with ENSA in the presence of vesicles, we observed a reduction in membrane-induced aggregation of A30P and G51D aSyn. Co-expression of ENSA with A30P in primary midbrain cultures rescued A30P-induced dopaminergic toxicity. Our findings suggest a novel therapeutic strategy to prevent formation of neurotoxic aSyn aggregates through stabilizing membrane-bound aSyn. These results have led us to initiate a search for "membrane stabilizers" of aSyn via high-throughput screening. We have identified several exciting leads that inhibit membrane-induced aggregation. These membrane stabilizers could be a powerful tool to examine how membrane-induced aggregation plays a role in aSyn neurotoxicity.

Disclosures: D. Ysselstein: None. **M. Joshi:** None. **V. Mishra:** None. **A.M. Griggs:** None. **G.P. McCabe:** None. **L.A. Stanciu:** None. **C.B. Post:** None. **J. Rochet:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

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Title: Reduced cytosolic calcium caused by SERCA activation is an early and pathogenic event in the cellular stress caused by alpha-synuclein oligomers

Authors: *C. BETZER[1], L. BERKHOUDT LASSEN[1], M. BRINI[3], T. CALI[3], A. OLSEN[2], W.-P. GAI[4], J. ANDERSEN[1], P. JENSEN[1];
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Abstract: Accumulation of aggregated alpha-synuclein (AS) in degenerating neurons in Parkinson's disease (PD) represents the patho-anatomical hallmark. Epidemiological studies demonstrate a protective effect of brain penetrating calcium channel antagonists against development of sporadic PD in patients treated for hypertension, suggesting that aggregated AS leads to alterations in calcium homeostasis. We analyzed the cellular calcium levels in cell based models of AS aggregation dependent degeneration. The cytosolic calcium levels were measured in live cells by use of Fura-2 and high-content-fluorescence microscopy. The cell lines used were differentiated human neuroblastoma cells, rat oligodendroglia cells, and primary mouse hippocampal neurons. Measurement of cytosolic calcium in these cell models revealed an early decrease and a later increase in cellular calcium when degeneration occurs. Endoplasmic calcium ATPase, SERCA plays a critical role in maintaining normal cytosolic calcium by pumping excess cytosolic calcium into the endoplasmic reticulum. We demonstrate that soluble AS oligomers, in contrast to monomers, bind SERCA and activate the pump as measured *in vitro* by increased ATPase activity and transport of calcium. We hypothesize that the increased ATPase activity causes the early decrease in cellular calcium level in our cell models. To test this, we treated the cells with low doses of a reversible SERCA inhibitor, cyclopiazonic acid (CPA). This treatment normalized both the initial reduction in cellular calcium, but also the later increase, suggesting that there is a direct link between the two phenomena. Furthermore, we found that CPA treatment improved the viability in the cell based models, strengthening our hypothesis that the early decreased cellular calcium is harmful and if untreated will lead to increased cellular calcium and cellular degeneration. In conclusion, decreased cytosolic calcium is an early disease-propagating event in the course of AS oligomer cytotoxicity that can be pharmacologically targeted by inhibitors of SERCA and represents a novel therapeutic strategy to be tested in synucleinopathies like PD, multiple system atrophy, and dementia with lewy bodies.

Disclosures: C. Betzer: None. **L. Berkhoudt Lassen:** None. **M. Brini:** None. **T. Cali:** None. **A. Olsen:** None. **W. Gai:** None. **J. Andersen:** None. **P. Jensen:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

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Ente CR Firenze

MJFF RRIA2011

Title: The hyperpolarization-activated current as a determinant of selective nigrostriatal degeneration in Parkinson's disease

Authors: *G. MANNAIONI, R. NARDUCCI, F. RESTA, C. CARBONE, G. PROVENSI, A. COSTA, A. MASI;
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Abstract: Parkinson's Disease (PD) is caused by massive, selective degeneration of dopaminergic (DA) neurons in the Substantia Nigra pars compacta (SNc). In contrast, DA neurons in the neighbouring Ventral Tegmental Area (VTA) are much less affected. The bases of this peculiar aspect of the disease are still unclear, as the two populations share all the neurochemical and biophysical properties deemed critical in PD pathogenesis. Increasing evidence suggests that a complex SNc-specific interplay of pathogenic determinants, rather than individual factors, underlies selective vulnerability. We recently demonstrated that MPP+, a neurotoxin able to cause selective nigrostriatal degeneration in rodents and primates, alters the electrophysiological properties of SNc DA neurons *in vitro* by inhibiting the Hyperpolarization-activated current (Ih). In midbrain DA neurons from TH-GFP mice, we found that pharmacological suppression of Ih increases the amplitude and duration of evoked Excitatory Post-Synaptic Potentials (EPSP). Moreover, Ih suppression leads to temporal summation of multiple EPSPs, a result of the impaired ability to resolve individual excitatory synaptic inputs at somatic level. The extent of this response depends on postsynaptic Ih magnitude and is significantly greater in SNc compared to VTA DA neurons. These results indicate that Ih regulates dendritic excitability differentially within midbrain DA neurons and suggest that differential impact of MPP+-mediated Ih suppression may underlie selective vulnerability. In this respect, we have obtained preliminary experimental evidence that intranigral injection of Ih blockers recapitulates the DA degeneration pattern generated by MPP+. Overall, these findings support the hypothesis that Ih loss of function, possibly caused by PD-trigger mechanisms such as mitochondrial failure and oxidative stress, may act in concert with SNc-specific synaptic connectivity to promote selective vulnerability.

Disclosures: G. Mannaioni: None. **R. Narducci:** None. **F. Resta:** None. **C. Carbone:** None. **G. Provensi:** None. **A. Costa:** None. **A. Masi:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

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Program#/Poster#: 41.04/C38

Topic: C.03. Parkinson's Disease

Title: Effects of agricultural pesticides on synaptic function

Authors: *K. MYERS[1,2], C. MARTIN[3], C. AAMODT[2], D. E. KRANTZ[4], F. E. SCHWEIZER[1]; [1]Neurobio., David Geffen Sch. of Medicine, UCLA, Los Angeles, CA; [2]Interdepartmental PhD Program for Neurosci., [3]Interdepartmental PhD Program in Mol. Toxicology, [4]Dept. of Psychiatry and Biobehavioral Sciences, Hatos Ctr. For Neuropharmacology, David Geffe, UCLA, Los Angeles, CA

Abstract: The factors leading to onset of Parkinson's disease (PD) are unknown, although there is strong evidence linking environmental toxins and genetic factors to individual susceptibility. Zinc dimethyldithiocarbamate (ziram), a widely used fungicide, has been shown to increase the risk of PD in exposed farm workers and nearby residents (Wang et al. 2011). We previously found that mammalian neurons in primary culture display an increase in the frequency of miniature EPSCs and IPSCs following acute exposure to ziram. As ziram has been found to directly inhibit E1 ubiquitin activating enzyme, and thus indirectly the 20S proteasome, we examined whether other pesticides with links to PD or the ubiquitin signaling system could cause similar changes in synaptic transmission. Amongst the tested compounds, Maneb, also a dithiocarbamate, had effects similar to ziram and appeared to disrupt the E1 enzyme. We examined the intrinsic excitability of neurons acutely exposed to ziram and discovered changes in neuronal firing patterns and excitability. Intriguingly, using the *Drosophila* NMJ as a model, we found that aminergic neurons - which may be susceptible in PD - respond differently to ziram than do glutamatergic neurons. Our findings demonstrate that exposure to this class of drug causes robust changes in synaptic transmission, leading to neuronal dysfunction and possibly excitotoxicity.

Disclosures: K. Myers: None. **C. Martin:** None. **C. Aamodt:** None. **D.E. Krantz:** None. **F.E. Schweizer:** None.

Poster

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Title: Glycosylation of synaptic vesicle glycoprotein 2C (SV2C) affects vesicular packaging of dopamine

Authors: *K. STOUT[1], M. OZAWA[2], A. DUNN[2], C. HOFFMAN[2], M. WANG[2], G. MILLER[2]; [1]Envrn. Hlth., [2]Emory Univ., Atlanta, GA

Abstract: An isoform of the synaptic vesicle glycoprotein 2 (SV2A, B, or C) localizes to every neurosecretory vesicle. Though the exact function of these proteins remains unclear, it is postulated that they stabilize neurotransmitter packaging via chemiosmotic stabilization, a process in which glycan chains on the intraluminal loop bind neurotransmitter. This interaction is thought to reduce the neurotransmitter concentration gradient and allow increased vesicle packaging. While SV2A and B are ubiquitously expressed throughout the brain, SV2C has a distinct basal ganglia expression and co-localizes strongly with tyrosine hydroxylase, the rate-limiting enzyme of dopamine synthesis. Given these data, we hypothesized that glycosylation of SV2C increases vesicular packaging of dopamine. To address this hypothesis, we generated site-directed mutants of each of the five N-glycosylation sites of SV2C and expressed them in human embryonic kidney (HEK) cells. We thoroughly characterized glycosylation via western blotting and immunocytochemistry. As certain forms of glycosylation are indispensible for protein trafficking, we visualized protein localization via total internal reflection fluorescence microscopy of immunolabelled cells. No individual glycan mutation affected protein localization. To address the functional changes of the mutated proteins we used false fluorescent neurotransmitter 206 (FFN206). FFN206 is a pH-sensitive fluorophore with affinity for the vesicular monoamine transporter 2 (VMAT2). HEK cells stably expressing human VMAT2 were transfected with pcDNA, SV2C, or site-directed mutants and the resulting uptake of FFN206 measured. Fluorescence experiments were confirmed with radioactive dopamine uptake assays. These data provide the first evidence of SV2C induced modulation of dopamine packaging.

Disclosures: K. Stout: None. **M. Ozawa:** None. **A. Dunn:** None. **C. Hoffman:** None. **M. Wang:** None. **G. Miller:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Title: SSAO/VAP-1 is altered in Parkinson's disease: possible role of the vascular system

Authors: ***M. SOLE**[1], M. UNZETA[1], T. VALENTE[2];
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Abstract: Increasing evidences suggest that cerebrovascular system dysfunction may play a role in the onset and progression of neurological diseases. Semicarbazide-sensitive amine oxidase (SSAO) is an enzyme highly expressed in brain vasculature and also released into blood. Its expression and activity are altered in neuropathological conditions, in which progression it is believed to contribute by releasing toxic products through its catalytic activity (Solé et al., Biochim Biophys Acta 2008), and by promoting the leukocytes binding and transmigration to inflamed tissues, acting as vascular adhesion protein 1 (VAP-1) (Jalkanen and Salmi, Blood 2007). Actually, an increase of its soluble plasmatic form is observed in multiple sclerosis (MS) (Airas et al., J Neuroimmunol 2006), in stroke, where it predicts the neurological outcome (Hernandez-Guillamon et al., Stroke 2010; Hernandez-Guillamon & Solé et al., Cerebrovasc Dis 2011), and in severe Alzheimer's disease (AD) (del Mar Hernandez et al., Neurosci Lett 2005), where it reflects the elevated membrane-bound form (Ferrer et al., Neurosci Lett 2002). Some pathological markers are shared among neurodegenerative diseases, and there is nowadays a great interest in identifying them for therapeutic purposes. In this regard, it is known that Parkinson's disease (PD) patients have vascular risk factors and display cerebrovascular abnormalities including microbleeds and white matter lesions, which is classified as vascular parkinsonism (VP) (Korczyn, Nat Rev Neurol 2015). These evidences suggest that vascular lesions can contribute to cognitive impairment in PD. However, this is a poorly studied issue, and precise changes in blood vessels have not yet been described in detail. In addition, there exists a clear inflammatory component in PD including peripheral immune cell infiltration (Chao et al., Biomed Res Int 2014). Therefore, our objective has been to determine the levels of SSAO/VAP-1 expression and activity in samples from PD patients in order to evaluate it as possible biomarker, pathologic mechanism and/or therapeutic target overlapping between several neurological disorders. We have observed an altered expression of SSAO/VAP-1 in different brain regions of PD patients compared to control samples. These results suggest the involvement of the vascular system in PD, although more studies should be performed to determine if this is a consequence of the neurodegeneration (cerebrovascular dysfunctions and/or pathologic

alterations in the blood-brain barrier) or could also contribute to the disease progression. These results will allow designing new SSAO inhibitors for therapeutic purposes.

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Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH F31

Title: Global profiling of HSP90 complexes in human pluripotent stem cell-derived midbrain dopamine neurons

Authors: ***S. KISHINEVSKY**[1], J.-W. SHIM[3], W. TAI[2], E. MOSHAROV[4], A. RODINA[2], J. PHILLIP[2], S. CHUNG[2], T. TALDONE[2], M. ALPAUGH[2], A. KRUG[5], S. GUTBIER[5], A. KAVALIER[6], T. MILNER[6], M. LEIST[5], S. GROSS[6], H. ERDJUMENT-BROMAGE[2], R. HENDRICKSON[2], G. CHIOSIS[2], L. STUDER[2];
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Abstract: A feature common to many neurodegenerative diseases is the disruption of protein homeostasis. HSP90 co-chaperone complexes stabilize protein interactions in many cells and adapt to different signaling events during disease progression. However, changes in protein homeostasis during early neurodegenerative events have not been elucidated. Neurons differentiated from induced pluripotent stem cells (iPSCs) make it possible to directly examine the cells involved in neurodegenerative diseases. Here, we leverage iPSC technology and chemical tools to investigate how HSP90 complexes could propagate Parkinsonian-relevant signaling cascades. We examine global HSP90 complex changes in iPSC-derived midbrain neurons in response to Parkinson's-relevant stress. We identified HSP90 complex alterations that resulted in increased oxidative stress and neurodegeneration. Hsp90 inhibition partially reversed this cascade. Our findings suggest a mechanism by which dysfunctional chaperone protein-mediated "stress" networks can inflict and perpetuate cell-type specific damage in Parkinson's disease.

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Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

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Title: Disruption of the synaptic vesicle glycoprotein 2C (SV2C) in Parkinson's disease

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Abstract: The synaptic vesicle plays two important roles in dopamine neurons by packaging transmitter to prepare for neurotransmission, and by sequestering cytosolic toxicants from the rest of the cell. Impaired storage of dopamine is a characteristic of Parkinson’s disease (PD) and genetic mutations in the vesicular monoamine transporter 2 (VMAT2) lead to parkinsonism. Enhancement of vesicular function through increased expression of VMAT2 in either mice or humans confers resistance to dopamine neuron degeneration. Furthermore, autosomal dominant mutations in other vesicle-associated proteins, such as α-synuclein and LRRK2, cause PD. These data suggest that characterizing additional modulators of dopamine vesicle function may be important in further studying and identifying potential therapeutic targets in PD. Polymorphisms upstream of the synaptic vesicle glycoprotein 2C (SV2C), a vesicular protein enriched in the basal ganglia, were recently shown to mediate the protective effect of smoking against PD,

suggesting an important role for SV2C in dopaminergic neurons. While the exact molecular function of SV2C is unknown, it likely positively modulates vesicular function and may represent a novel mediator of basal ganglia neurotransmission. However, the role of SV2C protein in PD has not been previously investigated. Here, we present immunohistochemical data detailing SV2C's expression in human striata in PD, other neurodegenerative diseases, and in age-matched control cases. SV2C localizes to dopaminergic terminal regions and GABAergic cells in the striatum in humans and mice. SV2C expression is specifically and dramatically disrupted in PD but preserved in other neurodegenerative diseases. In PD, SV2C expression is punctate in an apparent aggregate-like pattern throughout the striatum. A similar disruption in SV2C staining is observed in the striata of animals overexpressing PD-associated A53T α-synuclein under a Pitx3 (dopamine-neuron specific) promoter. Immunoprecipitation studies demonstrated a physical interaction between SV2C and α-synuclein, strengthening the association of α-synuclein with SV2C. SV2C also has potentially significant therapeutic relevance, as its close family member SV2A is the specific target for the antiepileptic compound levetiracetam. The results from our experiments indicate that SV2C may represent a novel pathology in PD, and that an interaction with SV2C may underlie this pathology.

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Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Title: A computational model of protein handling pathways in Parkinson's disease using SEED

Authors: *B. BEHROUZ, J. J. MORRISON, J. W. RYAN, A. M. GREEN, L. SCHAPPELL, K. S. INMAN, A. D. LEE;
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Abstract: While the cause of the neurodegeneration in Parkinson's Disease remains unknown, breakthrough discoveries of multiple genetic mutations provide clues into PD pathogenesis. These discoveries implicate biological pathways that are complex and heavily interconnected, making it insurmountable to decipher a clear picture of the downstream pathological impacts of each molecular change or interaction such as a genetic mutation. We utilized Simulation Environment for Experimental Design (SEED), a computing platform for virtual

experimentation, to create a preliminary in silico model of molecules and interactions implicated in PD. We focused on cellular protein handling mechanisms and genetic mutations such as VPS35. Within this system we have created multiple models based on gene knock-down and over-expression. These in silico models are a great complement to other *in vivo* and *in vitro* models with the advantage of spatio-temporal acuity. By visualizing these reaction chains in a unified system we are able to shed light on converging pathways that may be targeted for therapies with a higher likelihood of efficacy.

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Poster

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NSF 1156692.

Title: Antagonistic signaling mediated by the nuclear factor-kappa B and p38 regulates dopaminergic apoptotic cell death induced by gene (alpha [α]-synuclein)-environment (paraquat and manganese) interactions

Authors: A. ANANDHAN, P. HERNANDEZ-FRANCO, R. M. FOGUTH, *R. FRANCO; Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: The multifactorial etiology of Parkinson’s disease (PD) involves genetic, environmental, and aging risk factors. The pathological hallmarks of PD are the loss of dopaminergic neurons in the substantia nigra, and the accumulation of inclusion bodies (Lewy bodies) of aggregated alpha(α)-synuclein. Gene multiplications or point mutations in α-synuclein are associated with sporadic and familial PD. In addition, several reports have demonstrated that the toxicity of α-synuclein is modulated by pesticides, metals, mitochondrial toxins and pro-oxidant conditions. However, there is little consensus regarding the molecular mechanisms involved. We found that overexpression of wild type (WT) and mutant A53T α-synuclein exerts a toxic synergism in N27 dopaminergic cells exposed to the structurally unrelated environmental

toxicants paraquat and manganese (Mn). This toxic synergism was dependent on the activation of the mitogen/stress-activated protein kinase (MAPK/SAPK) p38, as evidenced by its inhibition with SB203580, and was not replicated in lung-derived A549 cells or neuroblastoma cells with low levels of tyrosine hydroxylase (TH). WT or A53T α-synuclein overexpression increased the phosphorylation (pSer536) of the nuclear factor kappa light-chain-enhancer of activated B cells (NF-κB) in mouse substantia nigra (AAV-mediated delivery), and in N27 dopaminergic cells, where it was also potentiated by paraquat and Mn exposure. However, the transcriptional activity of NF-κB was inhibited under the same experimental conditions. Ascorbic acid and inhibition of inducible nitric oxide synthase (L-NAME and 1400W) activity reduced the toxicity of α-synuclein and paraquat. Neither inhibition of the apoptosis signal-regulating kinase 1 (ASK1) or c-Jun N-terminal kinases (JNK) with NQDI-1 or SP600125, respectively, modulated the toxicity of α-synuclein and environmental agent exposure. These results suggest that while p38 exerts a pro-apoptotic, NF-κB acts as an anti-apoptotic signaling against of dopaminergic cell death induced by gene (alpha (α)-synuclein)-environment (paraquat and manganese) interactions.

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Poster

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Title: Testosterone increases oxidative stress-induced neuroinflammation in dopamine neurons

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Abstract: Neuroinflammation and oxidative stress are hallmarks of neurodegeneration in dopamine neurons in Parkinson’s disease (PD). Interestingly, men have a two-fold risk for PD than women. While the mechanisms underlying this sex difference remains elusive, one possibility may be that testosterone, the primary male sex hormone, is involved in dopamine

neurodegeneration. Our previous studies show that testosterone increases oxidative stress and dopamine neuronal death. A possible mechanism involved in the negative effects of androgens, may include the pro-inflammatory mediators NFkB and COX2. These mediators are responsive to oxidative stress and can increase alpha synuclein protein expression. Alpha synuclein is associated with Lewy bodies and apoptosis. Therefore, we hypothesize that under oxidative stress conditions, testosterone will increase inflammatory mediators NFkB and COX2, exacerbating apoptotic cell death in dopamine neurons. To test our hypothesis, we exposed a dopaminergic cell line (N27 cells) to a sublethal concentration of the pro-oxidant, tert-butyl hydrogen peroxide (H2O2) and assessed the role of testosterone on oxidative stress, cell viability and pro-inflammatory markers. Under oxidative stress conditions, there is a decrease in dopamine cell viability and an increase in NFkB, COX2, alpha synuclein and apoptosis. Interestingly, these factors are exacerbated by testosterone exposure. Further, inhibition of either NFkB or COX2 signaling can block testosterone's negative effects on cell viability. Thus, our data shows that testosterone may mediate the sex differences observed in PD by increasing oxidative stress induced neuroinflammation and apoptosis in dopamine neurons.

Disclosures: S. Holmes: None. **R.L. Cunningham:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Title: Characterization of monocyte sub-populations in PD patients and different mouse models for PD

Authors: *C. BLIEDERHAEUSER[1], V. GROZDANOV[1], L. ZONDLER[1], P. J. MCLEAN[2], F. GILLARDON[3], A. C. LUDOLPH[1], J. H. WEISHAUPT[1], K. M. DANZER[1];
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[3]Boehringer Ingelheim, Biberach an der Riss, Germany

Abstract: Parkinson's disease (PD) is a multisystem neurodegenerative disorder. Emerging evidence points to a contribution of inflammatory processes to the pathogenesis of PD but implications of the peripheral immune system are still poorly understood. To investigate whether the composition of myeloid cells is altered in PD, we applied a FACS based approach to characterize different monocyte sub-populations from PD patients and age-matched healthy controls. Strikingly, we found a strong increase in the ratio of classical CD14+ to non-classical

CD16+ monocytes. Comparing total numbers of monocytes from PD patients and healthy controls revealed no significant differences. The changes in the monocyte ratio in the peripheral blood of PD patients compared to healthy donors are attributed to a strong enrichment of classical CD14+ monocytes, and concurrently, a reduction of the non-classical CD16+ monocytes in PD. Interestingly, similar results could be found in two different transgenic mouse models for PD. Equivalent to human peripheral blood monocytes classical and non-classical monocytes in mice can be distinguished based on their expression of Ly6C. In detail, human classical CD14+ and non-classical CD16+ monocytes correspond to Ly6Chi and Ly6Clo in mice. Notably, we found an increased ratio of classical to non-classical monocytes in LRRK2(R1441G)-overexpressing mice as well as in a Thy-1 αsyn-wt model. These results suggest that different PD mouse models have a deregulation of monocyte sub-populations as a common feature. Moreover, we saw the strongest effect in monocyte deregulation in the LRRK2(R1441G) mouse model. Therefore, we asked whether LRRK2 might be differentially expressed in immune cells in PD patients. Using FACS analysis and quantitative RT-PCR we found so far increased levels of LRRK2 in B-cells of PD patients and increased LRRK2 levels in PD lymphoblasts compared to healthy controls, respectively. Taken together, our data suggest that deregulation of monocyte sub-populations plays an important role in PD and that LRRK2 potentially contributes to immune dysfunction. Identifying the role of the peripheral immune system in PD and additionally the role of LRRK2 in different immune cells during PD may pave the way for new therapeutic strategies in PD.

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Poster

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Topic: C.03. Parkinson’s Disease

Title: Inflammatory response to alpha-synuclein by monocytic cells from healthy controls and patients with Parkinson's disease

Authors: *V. GROZDANOV, C. BLIEDERHAEUSER, W. P. RUF, R. LANGOHR, L. ZONDLER, A. C. LUDOLPH, J. H. WEISHAUPT, K. M. DANZER;
Univ. of Ulm, Ulm, Germany

Abstract: Accumulating evidence suggests a role for the innate immunity in the pathogenesis of Parkinson's disease (PD). We have previously characterized peripheral blood monocytes from PD patients and compared them to healthy controls. We found an inflammatory dysregulation on transcriptome and functional level, including increased cytokine release upon inflammatory stimulation with lipopolysaccharide (LPS) and impaired phagocytic capacity. It has also been previously found that pathological alpha-synuclein (asyn) species can be found in the extracellular space, where they can possibly interact with immune cells. To investigate the immune response of myeloid innate immune cells to extracellular asyn, we studied the inflammatory response of monocytes and monocyte-derived macrophages to different forms of extracellular asyn. We found that pathological alpha-synuclein oligomers induce a robust cytokine response by monocytes. This inflammatory response is even potentiated by familial mutations of alpha synuclein. Furthermore, we found that the pathological hyperactivity of PD monocytes in response to LPS is also found in response to extracellular asyn. Taken together, our data indicates that pathological asyn species trigger an inflammatory response by myeloid innate immune cells and suggest that monocytes infiltrating the CNS in the course of PD can contribute to tissue damage by immune response to extracellular asyn.

Disclosures: V. Grozdanov: None. **C. Bliederhaeuser:** None. **W.P. Ruf:** None. **R. Langohr:** None. **L. Zondler:** None. **A.C. Ludolph:** None. **J.H. Weishaupt:** None. **K.M. Danzer:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 41.14/C48

Topic: C.03. Parkinson’s Disease

Support: MJF

Title: Content of alpha-synuclein in erythrocyte-derived microvesicles: implications for Parkinson’s disease

Authors: *J. LAMONTAGNE-PROULX[1], I. ST-AMOUR[1], N. CLOUTIER[2], G. CISBANI[1], K. COULOMBE[1], S. MASON[4], A. HUBERT[2], C. GILBERT[2], N. DUPRÉ[5], M. LANGLOIS[5], S. LACROIX[3], R. BARKER[4], É. BOILARD[2], F. CICCHETTI[1];
[1]Neurosciences, [2]Microbiologie-infectiologie et immunologie, [3]Médecine moléculaire, Ctr. De Recherche Du CHUL, Quebec, QC, Canada; [4]Clin. Neurosciences, John van Geest Ctr. for Brain Repair, Cambridge, United Kingdom; [5]Neurosciences, Hôpital de l'Enfant-Jésus, Quebec, QC, Canada

Abstract: Accumulating evidence points to an abnormal immune response and blood-brain barrier dysfunctions as contributing factors to the pathogenesis of Parkinson's disease (PD). In the blood, α-synuclein (αSyn) is expressed in platelets, leukocytes and erythrocytes, with levels reported to correlate with disease duration (Pienimaeki-Roemer A and al. 2015; Barbour R and al. 2008; Colasanti T and al. 2014). In normal physiological condition, the production of extracellular vesicles (EV) such as microvesicles (MV) and exosomes is a property of most eukaryotic cells which may, however, be precipitated in a pathological context. The aim of our study was to quantify EV in the plasma of PD patients and assess their relevance as a potential biomarker of the disease. Using a FACS Canto II with a forward scatter coupled to a photomultiplier tube, we quantified cell-derived MV from platelets, erythrocytes, monocytes and endothelial cells in platelet-free plasma (PFP) from 59 PD patients and 37 age and sex-matched Controls. The concentrations of these cell-derived MV were similar between PD patients and Controls, with high concentrations of erythrocyte-derived CD235+ MV (EMV) (7.01 ± 1.7 x 108 and 9.83 ± 2.1 EMV/ml of PFP in PD patients and Controls, respectively, P=0.32). Positive correlations were identified between EMV concentrations and disease progression based on both the unified Parkinson's disease rating scale and Hoehn & Yahr scores (R2= 0.30 and 0.17 respectively, P<0.05). Exosomes and EMV were further isolated from erythrocytes following a treatment with calcium ionophore A23187 and sequential centrifugations, and the concentration of αSyn was quantified using ELISA assays. The mean concentration of αSyn was 1004 ± 128ng and 1028 ± 273ng in 100 µl of packed erythrocytes and 4.24 ± 0.54ng and 4.44 ± 1.01ng in EMV, in PD patients and Controls respectively (P=0.88). The concentration of αSyn in exosomes was similar with values of 0.21 ± 0.01 and 0.43 ± 0.23ng (PD vs. Control, P=0.96). Two-Way ANOVA analyses revealed higher concentration of αSyn in EMV as compared to exosomes (P<0.0001). This correlation unveils EMV as a potential non-invasive biomarker of disease progression as well as for monitoring drug efficacy. The presence of αSyn in EMV and exosomes may further point to the potential role of erythrocyte-derived EV in α-synucleinopathies. Further studies to investigate the mechanisms responsible for the erythrocyte-related EV release in PD and the specificity of EMV counts as a biomarker are currently underway.

Disclosures: J. Lamontagne-Proulx: None. **I. St-Amour:** None. **N. Cloutier:** None. **G. Cisbani:** None. **K. Coulombe:** None. **S. Mason:** None. **A. Hubert:** None. **C. Gilbert:** None. **N. Dupré:** None. **M. Langlois:** None. **S. Lacroix:** None. **R. Barker:** None. **É. Boilard:** None. **F. Cicchetti:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 41.15/C49

Topic: C.03. Parkinson's Disease

Support: DA034783

Title: Park2-/- rats have a decreased ratio of tetrameric to monomeric alpha-synuclein in the striatum

Authors: *A. MOSZCZYNSKA[1], B. A. KILLINGER[2], L. XU[2], A. DUTTA[2];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by the progressive loss of dopamine neurons in the midbrain and the formation of alpha-synuclein positive inclusions termed "Lewy bodies." The molecular details of alpha synuclein aggregation *in vivo* are unclear. It is well known that excess monomeric alpha-synuclein readily aggregates; however alpha-synuclein *in vivo* forms a stable tetramer which is not known to aggregate. Loss of parkin function due to mutation of Park2 gene is a characteristic feature of familial PD. Here we tested the hypothesis that the stoichiometry of alpha-synuclein species would be altered in Park2-/- rats. Using a combination of blue native electrophoresis and chemical cross-linking we developed a straight forward method to measure alpha-synuclein stoichiometry in Triton x-100 soluble tissue samples that circumvent some of the issues associated with tissue cross-linking. We then used this method to measure the ratio of stable alpha-synuclein species in our PD model. In striatal lysates from Park2-/- rats native alpha-synuclein occurred as three detectable species; ~16 kDa (monomer), ~28 kDa (dimer), and ~56 kDa (tetramer). The apparent monomer was the most abundant species with the dimer and tetramer being less abundant. Recombinant alpha-synuclein also formed stable dimer and teramer when incubated for several days at 4°C. The recombinant alpha-synuclein also appeared to form disordered aggregate like species, which were not present *in vivo*. The ratio of stable alpha-synucelin tetramer to monomer decreases in the striatum of Park2-/- rats as compared to wild-type rats. This finding suggests that decreasing the ratio of tetramer to monomer may promote dysfunction in striatal neurotransmission and dopamine neuron neurodegeneration. Future studies should determine the biological function of native monomeric and tetrameric alpha-synuclein.

Disclosures: A. Moszczynska: None. **B.A. Killinger:** None. **L. Xu:** None. **A. Dutta:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 41.16/C50

Topic: C.03. Parkinson's Disease

Support: UNAM PAPIIT IA202214-2

FESI-DIP-PAPCA-2014-18

UNAM PAPIIT IN2151114

FESI-DIP-PAPCA-2014-16

Title: L-DOPA as an inducer of reactive oxygen species in different brain structures

Authors: A. L. CASTRO CRUZ[1], *J. RAMOS[2], M. T. IBARRA-GUTIERREZ[1], A. L. GUTIERREZ-VALDEZ[1], M. AVILA-COSTA[1], V. ANAYA-MARTINEZ[1];
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Abstract: Parkinson's disease (PD) is characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), causing the decrease of dopamine levels in the innervating nuclei and producing impaired of motor activity. Various treatments have been proposed to counteract the symptoms, L-dopa is the most common drug for the treatment of PD because of its clinical efficacy; however, long-term L-dopa treatment induces involuntary abnormal movements, several evidences suggest that L-dopa can increase the oxidative stress pre-existing condition. However; some studies reported no damage, and shows protective action against free radicals. Therefore the aim of this study was to evaluate if the levodopa can cause some damage in healthy rats using the analysis of lipid peroxidation in several brain structures (SNc, striatum, motor cortex, hippocampus and globus pallidus). The Levodopa/carbidopa (10mg/1mg/kg) were administered orally daily for a 3 months (n=12) in rats, lipid peroxidation was determined in each cerebral structure by the technique of TBARS. Significant differences from controls were found in the globus pallidus, however, it was also evident the tendency to increase in the striatum and SNc. This data demonstrate that L-dopa induce lipid peroxidation.

Disclosures: A.L. Castro Cruz: None. **J. Ramos:** None. **M.T. Ibarra-Gutierrez:** None. **A.L. Gutierrez-Valdez:** None. **M. Avila-Costa:** None. **V. Anaya-Martinez:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: NIH/NINDS R01 #NS085070

NIH/NINDS P50 #NS072187

Title: Exploring the potential of phospho-ubiquitin antibodies as a biomarker tool for aging and disease

Authors: *F. C. FIESEL, M. CASTANEDES-CASEY, D. DICKSON, Z. WSZOLEK, P. MCLEAN, H. MELROSE, W. SPRINGER;
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Abstract: PINK1, a kinase involved in recessive familial forms of Parkinson's disease (PD), senses mitochondrial stress and is required to induce the degradation of damaged mitochondria (mitophagy). PINK1-dependent phosphorylation of Parkin at Ser65 in its N-terminal ubiquitin-like domain has been shown to be important to recruit and activate the E3 ubiquitin ligase to mitochondria. Parkin ubiquitinates a variety of different mitochondrial substrates. Subsequently, mitochondrial proteins are degraded by the ubiquitin proteasome pathway or lysosomal autophagic degradation. Recently, it has been discovered that PINK1 also phosphorylates ubiquitin itself at the conserved Ser65 position. This event is thought to enhance Parkin activation and mitochondrial degradation. We have generated two antibodies that are specific for the phosphorylated variant of ubiquitin. We have validated the antibodies using fibroblasts from controls and PINK1 mutation carriers. Since mitochondrial dysfunction plays a role for the pathogenesis of PD as well as other age-related diseases and the aging process itself, we will test our antibodies as a tool to characterize mitochondrial stress *in vivo*. Using brain sections from young, old and diseased individuals, we will analyze the burden of phospho-ubiquitin in different brain regions. We use classical IHC methods combined with state-of-the-art digital image analysis to analyze the abundance of phospho-ubiquitin. Our data shows a granular pattern of phospho-ubiquitin that partially colocalizes with mitochondria and lysosomes. In addition, we will analyze brains from mice that overexpress alpha-synuclein or express mutant LRRK2 and are used to model autosomal dominant forms of PD. Altogether, this study will address a potential functional overlap between different forms of familial and sporadic PD and will determine the suitability of phospho-specific ubiquitin antibodies as tools to detect mitochondrial stress in aging and disease.

Disclosures: F.C. Fiesel: None. **M. Castanedes-Casey:** None. **D. Dickson:** None. **Z. Wszolek:** None. **P. McLean:** None. **H. Melrose:** None. **W. Springer:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: Massachusetts' Alzheimer's Disease Research Center and the Harvard NeuroDiscovery Center

National Institute of Neurological Disorders and Stroke R21NS067335

Title: miRNAs and their involvement in the pathology of dopamine neurons in Parkinson's disease

Authors: *K. C. SONNTAG[1], C. E. BRIGGS[2], T.-U. W. WOO[1], L. K. IYER[3]; [1]Psychiatry, McLean Hospital/Harvard Med. Sch., Belmont, MA; [2]The Ctr. of Cancer Systems Biol., [3]Dept. of Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: The degeneration of substantia nigra (SN) dopamine (DA) neurons in sporadic Parkinson's disease (PD) is characterized by disturbed gene expression networks. Micro(mi)RNAs are post-transcriptional regulators of gene expression and there is increasing evidence that they are involved in the molecular pathogenesis of neurodegenerative disorders, including PD. Here, we document a comprehensive analysis of miRNAs in SN DA neurons from postmortem brains of PD patients and healthy controls. Our data show that miRNAs are dysregulated in disease-affected neurons and are differentially expressed between male and female samples with a trend of more up-regulated miRNAs in males and more down-regulated miRNAs in females. Unbiased Ingenuity Pathway Analysis (IPA) revealed a network of miRNA/target-gene associations that is consistent with dysfunctional gene and signaling pathways in PD pathology. Based on our findings from the miRNA profiles and computational data analysis, we started to functionally analyze miRNAs of interest. To this end, we studied miR-126, which was upregulated in PD and has been implicated in regulating Insulin/IGF-1/PI3K signaling, a pathway that was also dysregulated in the PD DA neurons. Our data show that miR-126 may play a profound role in neuronal cell survival to toxic insult by regulating growth factor (GF)/PI3K/AKT and MAPK/ERK signaling cascades. Altogether, our data provide evidence for an association of miRNAs with the cellular function and identity of neurons in general and SN DA neurons in particular, and with deregulated gene expression networks and signaling pathways related to neurodegenerative diseases, including PD, that may be sex-specific. Further functional characterization of miRNA/target gene relationships in neurons may lead to the identification of novel therapeutic targets for the treatment of neurological and age-related disorders.

Disclosures: **K.C. Sonntag:** None. **C.E. Briggs:** None. **T.W. Woo:** None. **L.K. Iyer:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 41.19/C53

Topic: C.03. Parkinson's Disease

Support: CONACYT-169023

VIEP 2014-2015

Title: Differential striatal FosB and ΔFosB mRNA expression after acute, sub-chronic or chronic levodopa treatment in a rat model of Parkinson´s disease

Authors: ***V. PALAFOX**[1], V. SOSTI[2], J. KULISEVSKY[2], J. AGUILERA[3], I. LIMON[1]; [1]Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [2]Laboratori de Neuropsicofarmacologia, Inst. de Recerca de l´Hospital de la Santa Creu I de Sant Pau, Barcelona, Spain; [3]Dept. de Bioquímica i de Biología Mol. and Inst. de Neurociències,, Barcelona, Spain

Abstract: In Parkinson´s diseases the best therapy is Levodopa, unfortunately after chronic treatment almost all patients developed dyskinesia, which are very disability motor disorder. The over expression of transcription factor FosB and ΔFosB after chronic levodopa treatment is considered the molecular mechanisms that are involved in the dyskinetogenic process. The aim in this study was evaluated the responses of striatal FosB and ΔFosB mRNA expression after acute, sub-chronic or chronic levodopa treatment in a rat model of Parkinson´s disease. The Sprague Dawley rats received unilateral 6-OHDA-injection in the medial forebrain bundle by stereotactic surgery. At eight day post-surgery the rats were evaluated in the cylinder test to determinate the degree of dopaminergic damage, two days after, rats received acute (single dose), sub-chronic (three dose /three days) or chronic (sixteen dose/sixteen days) levodopa (10 mg/kg i.p) treatment. Rats were decapitated to obtain the brain and was dissected the striatum 1 h, 3 h and 24 h after the last levodopa injection by each challenged levodopa. We determinate both isoforms FosB and ΔFosB mRNA by real time-PCR in the striatum. The results showed that single dose levodopa induces high fold ΔFosB and FosB mRNA at 1 h post-injection of the drug, while that 24 h after the fold induction of FosB and ΔFosB mRNA are very similar like control group. However, after sub-chronic levodopa treatment the fold induction of FosB and ΔFosB mRNA decreases with respect to acute treatment although is possible observed a little induction,

it is not significant. However after chronic levodopa the fold induction of striatal FosB and ΔFosB mRNA are again recuperate at three hours post-injection, although it is no the same magnitude as in acute treatment. In summary, this pattern of FosB and ΔFosB mRNA expression result reflecting neuronal response since the first levodopa dose although dyskinesia are expressed during the chronic levodopa treatment. Palafox-Sanchez V. received a scholarship of Mexico government (CONACYT, 366871) Support: VIEP (2014-2015), CONACYT

Disclosures: **V. Palafox:** 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-MEXICO, VIEP-BUAP. **V. Sosti:** None. **J. Kulisevsky:** None. **J. Aguilera:** None. **I. Limon:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 41.20/C54

Topic: C.03. Parkinson’s Disease

Support: PICT 2011-521 (MGM), Argentina

UBACyT 2012-2015 (LR), Argentina

CONICET (CT), Argentina

IBRO Travel Grant (CT)

Title: Kv1.3 mediates cholinergic hyperexcitability in a mouse model of Parkinson's disease

Authors: ***C. TUBERT**[1], I. R. E. TARAVINI[2], G. SANCHEZ[3], A. PROST[1], L. RELA[1], M. G. MURER[1];
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Abstract: Balanced actions of dopamine (DA) and Acetylcholine (ACh) shape striatal function. In Parkinson´s disease (PD) this balance is lost, leading to an hypercholinergic state. ACh is released in the striaum by tonically active interneurons (ChIs). Recent work shows that ChIs are hyperexcitable in a rat model of PD as a result of a lack of “accommodation”, an intrinsic

property that markedly slows firing during sustained current injection. Here our aim is to identify currents that regulate ChIs accommodation in mouse brain slices. ChIs represent less than 5% of the striatal neurons, so we used mice expressing *tdTomato* fluorescent protein in ChIs (ChAT-Cre;rt) to find them easily. By means of immunohistochemistry we demonstrate that all *tdTomato* cells express ChAT in ChAT-Cre;rt mice, but not the markers of other striatal interneurons including PV, NPY and NOS. Moreover, *tdTomato*-ChIs have electrophysiological properties similar to those of ChIs recorded from wild type mice. Extending previous studies showing more excitable ChIs in juvenile mice, we found that ChIs almost always show accommodation in adult mice but many ChIs do not in juvenile animals. Thus, accommodation is developmentally regulated. Margatoxin (MgTx), a blocker of Kv1.3 channels, markedly attenuated accommodation in ChIs, shown by an increase in the number of spikes fired (from 7.2±1.3 to 13.3±1.1 spikes; p=0.003) and a prolongation of firing (from 332.2±127.2 to 828.8±55.6 s; p=0.008) during a 1s depolarizing current step. Dendrotoxin, a less selective Kv1 blocker, also reduced accommodation, while blockers of Kv7 channels like XE991 and UCL2077, were ineffective. MgTx also increased spontaneous firing (p=0.0455) measured in cell-attached recordings. We have isolated and characterized the MgTx-sensitive current in accommodating ChIs (V_{act}=-38$\pm$7mV, V_{50}=-3$\pm$8mV, I_{max}=1500pA). Immunohistochemistry in brain sections and PCR of laser dissected ChIs revealed the expression of Kv1.3 channels. Thus, ChIs express a functionally relevant Kv1 conductance. We have then evaluated the influence of endogenous DA on accommodation. Bath application of D_1 (SCH23390) and D_2 (sulpiride) type receptor antagonists does not block accommodation. However, as it is the case in rats, fewer ChIs show accommodation in a mouse model of PD induced with 6-OHDA. Moreover, MgTx-sensitive currents are smaller in ChIs of 6-OHDA-lesioned mice compared to sham-mice ($p<0.0001$). Our data indicate that chronic nigrostriatal lesions reduce the MgTx-sensitive current in ChIs, causing their lack of accommodation and hyperexcitability, and nominate Kv1.3 channels as potential new targets of antiparkinsonian therapy.

Disclosures: **C. Tubert:** None. **I.R.E. Taravini:** None. **G. Sanchez:** None. **A. Prost:** None. **L. Rela:** None. **M.G. Murer:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 41.21/C55

Topic: C.03. Parkinson’s Disease

Support: MUSC Barmore Foundation

Title: Vagus nerve stimulation activates pparγ as a mechanism to treat Parkinson's disease

Authors: *H. A. BOGER[1], R. GREGORY[1], K. HELKE[1], S. HAYS[2], V. HINSON[1], A. FARRAND[1];
[1]Neurosciences, Med. Univ. of South Carolina, Charleston, SC; [2]Univ. of Texas at Dallas, Dallas, TX

Abstract: Vagus nerve stimulation (VNS) is approved by the FDA for the treatment of drug-resistant epilepsy and depression. Although the exact mechanism is unknown, previous studies have shown increased firing rates of noradrenergic (NE) neurons in the locus coeruleus (LC), leading to increased brain derived neurotrophic factor (BDNF) levels in the LC and its target regions. Based on these previous findings, VNS makes for an intriguing treatment strategy for Parkinson's disease (PD) since LC-NE degeneration typically occurs prior to the dopaminergic (DA) loss of the substantia nigra (SN). Preliminary studies from our lab show that chronic VNS can attenuate the loss of tyrosine hydroxylase (TH)-positive neurons in the LC and SN observed in a double lesion model of PD, and restored the locomotor activity deficit caused by the lesion. A potential mechanism for this alleviation could be activation of the anti-inflammatory transcription factor, PPARγ, which can result in increased BDNF levels. Therefore, our hypothesis is that chronic VNS activates PPARγ to increase BDNF levels and attenuate the deficits observed in a double-lesion model of PD. We examined this hypothesis by utilizing a double-lesion rat model to mimic the deficits of PD. Adults male rats were administered the NE neurotoxin DSP-4 (50 mg/kg, ip) and after seven days underwent surgery to administer the DA neurotoxin 6-OHDA (6 μL, intrastriatal). Animals in the VNS group also got vagus cuff implants and head caps during the 6-OHDA surgery that could later be attached to a stimulator. Starting ten days post-lesion, the VNS animals were stimulated twice a day for two weeks using precise bursts of stimulation at a set amplitude and rate. Following the last stimulation, the rats were euthanized, the right frontal cortex, hippocampus, and dorsal striatum tissue samples were taken for ELISA detection of BDNF, and the left hemisphere was sectioned for immunohistochemical detection of TH, PPARγ, the BDNF receptor TrkB, and the pro-inflammatory marker COX-2. Rats in the VNS group have increased levels of BDNF in LC target regions (frontal cortex and hippocampus) and in the SN target region, dorsal striatum, compared to the double-lesion rats, as well as increased PPARγ expression in these areas. These data taken together imply that PPARγ activation leads to BDNF upregulation, and subsequently attenuates DA and NE lesion-induced deficits. Studies are ongoing in the laboratory to determine the relationship between PPARγ activation, BDNF levels, and TrkB expression following chronic VNS stimulation. Collectivley, these data point to an exciting potential treatment strategy for individuals with PD.

Disclosures: H.A. Boger: None. **R. Gregory:** None. **K. Helke:** None. **S. Hays:** None. **V. Hinson:** None. **A. Farrand:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 41.22/C56

Topic: C.03. Parkinson's Disease

Support: R01 NS038065

R01 NS086074

Title: p53-dependent regulation of autophagy by c-Abl in Parkinson's disease

Authors: *R. KARIM, E. LIAO, M. K. LEE;
Univ. of Minnesota, Minneapolis, MN

Abstract: Abnormal α-synuclein (αS) accumulation in neurons is a likely contributor to neurodegeneration in Parkinson's disease (PD). Recent studies show that PD is associated with the induction of c-Abl, a non-receptor tyrosine kinase that is activated by oxidative stress. Our studies show that activation of c-Abl in mouse model of α-synucleinopathy (*A53TαS*) is associated with activation of p53, indicating that c-Abl could promote cell death via p53. In addition, previous studies show that c-Abl activity could regulate autophagy and αS toxicity. Thus, we hypothesized that increase in c-Abl and/or p53 activation was responsible for the impaired autophagy-lysosomal process (ALP) seen in *A53TαS* Tg mice. To determine pathological significance of c-Abl in progressive α-synucleinoapthy, we treated the *A53TαS* mice with Nilotinib (Nilo), a FDA-approved c-Abl kinase inhibitor. Chronic administration of Nilo showed an attenuation of pathology and facilitates autophagic clearance of pathological αS. To determine how c-Abl inhibition alleviates ALP defect in *A53TαS* mice, we investigated whether p53 was sufficient to modulate ALP. Our results show that Nilo and p53 inhibitor, pifithrin-α (PFT), both increased autophagic flux in N2a and M17 cell lines. Moreover, Nilo and PFT activated ER-stress marker eIF2α, PDI and upregulated the phosphorylation of AMP-activated kinase (AMPK) which was accompanied by pS555-ULK1 activation and conversely down-regulated mTORC1 signal molecule ribosomal S6 and translation repressor protein 4EBP1. These results suggest that c-Abl regulates ALP via regulation of p53 and subsequent activation of ULK1 and inhibition of mTORC1 signal. Together our data reveal that Nilo-mediated autophagic clearance of toxic αS is possibly dependent on p53 and that treatment with Nilo may provide a therapeutic strategy for PD.

Disclosures: **R. Karim:** None. **E. Liao:** None. **M.K. Lee:** None.

Poster

041. Mechanisms of Cell Death and Dysfunction in Parkinson's Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS087559

NIH Grant DA033121

NIDA-IRP

Title: Acute administration of pramipexole alters Akt/GSK-3β signaling in the ventral pallidum

Authors: S. A. GRASSO[1], *A. L. PERSONS[1], S. E. TEDFORD[1], A. H. NEWMAN[2], T. C. NAPIER[1];
[1]Pharmacol., Rush Univ. Med. Ctr., Chicago, IL; [2]NIDA IRP, Baltimore, MD

Abstract: Pramipexole (PPX), a direct-acting dopamine agonist used in the treatment of Parkinson's disease and restless leg syndrome, is associated with the development of addiction-like behaviors such as problem gambling. PPX demonstrates high affinity for the D3 receptor (D3R) subtype, which may be involved in these behaviors, but the D3R-associated signaling mechanisms of PPX are largely unknown. D3Rs are pleotropic and can signal in a G protein-independent manner *via* a Akt/GSK-3β pathway. Stimulation of D3R activates protein phosphatase 2A (PP2A), which dephosphorylates Akt. Dephosphorylation of Akt reduces phosphorylation of constitutively active GSK-3β, a known mediator of addiction-related changes in the brain. The ventral pallidum (VP) is a limbic brain structure that regulates key aspects of addiction-associated behaviors, and it contains a high density of D3Rs; yet the nature of D3R signaling within the VP is largely unknown. We tested the hypothesis that acute PPX administration will decrease phosphorylation of Akt and GSK-3β in the VP. Rats were treated with saline or (±)PPX (4mg/kg), and 1hr post-injection, the VP was harvested. A Western blot protocol was used to determine levels of Akt and GSK-3β). Acute PPX reduced the ratio of pAkt/Akt (i.e., active/total) in the VP. Additionally, downstream of Akt, the ratio of pGSK-3β (inactive)/GSK-3β (total) VP was reduced. A 30 min pretreatment with PG01037, a D3R-selective antagonist, attenuated the PPX effects on both Akt and GSK-3β. These studies indicate that PPX-mediated activation of D3R alters signaling through Akt and GSK-3β in the VP. Neuroadaptive processes such as these may link PPX with the development of addiction-like behaviors.

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Poster

042. Huntington's Disease Clinical

Location: Hall A

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Program#/Poster#: 42.01/C58

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Center for Medical Systems Biology

EC7FP Project 261123

EC7FP Project 201413

EC7FP Project Neuromics

Title: Huntington’s disease biomarker progression profile identified by transcriptome sequencing in peripheral blood

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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that manifests itself through cognitive, psychiatric and motor symptoms. The pathology is caused by an expanded CAG repeat in the HTT gene, resulting in a mutant huntingtin protein. With several therapeutic approaches in development for Huntington’s disease, there is a need for easily accessible biomarkers to monitor disease progression and therapy response. We performed next generation sequencing-based transcriptome analysis, of total RNA from peripheral blood of 91 mutation carriers, (27 presymptomatic and, 64 symptomatic) and 33 controls. Transcriptome analysis by DeepSAGE identified 167 genes significantly associated with clinical total motor score in Huntington’s disease patients. Relative to previous studies this yielded both novel genes, and confirmed previously identified genes , such as H2AFY, an overlap in results which has proven difficult in the past. Pathway analysis showed enrichment of genes of the immune system and of target genes of miRNAs which are downregulated in Huntington’s disease models. Using a highly parallelized microfluidics array chip (Fluidigm) we validated 12 of the top 20 significant genes in our discovery cohort and 7 in a second independent cohort. The five genes (PROK2, ZNF238, AQP9, CYSTM1, and ANXA3) which were validated independently in both cohorts present a candidate biomarker panel for stage determination and therapeutic readout in Huntington’s disease. Our data support the view that peripheral blood is a useful source to

identify biomarkers for Huntington's disease and monitor disease progression in future clinical trials.

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Poster

042. Huntington's Disease Clinical

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Program#/Poster#: 42.02/C59

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: BN82451B in Huntington's disease: on the way to translational biomarkers

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Abstract: BN82451B is a small multitargeted orally active molecule which interferes with excitotoxicity (sodium channel blocker), oxidative stress (antioxidant) and inflammation (cyclooxygenase [COX] inhibitor) that is currently in Phase 2a in Huntington patients. In order to further support clinical development and to monitor pharmacological activity of this compound, we investigated potential target efficacy biomarkers in non-clinical samples, e.g. serum and whole blood in cynomolgus monkeys and serum, whole blood, urine and brain tissues in rats. In rats, oral treatment at 25 mg/kg/day during 4 days resulted in marked lower prostaglandin E2 (PGE2) levels in serum and urine. In brain tissues, measured PGE2 levels were reduced by 65% compared to controls in accordance with the BN82451B biodistribution (brain to plasma ratio of 3), demonstrating pharmacological activity of the compound in the central nervous system. In monkeys treated at 30mg/kg/day during 11 days, we confirmed PGE2 as a biomarker of BN82451B activity in the serum. Whole blood transcriptomic analysis in this species showed that treatment had an impact on gene expression highlighting 329 differentially expressed genes common to male and female monkeys ($p=0.05$, multiple test correction applied, 2 fold change cutoff). Regulated genes were either related to the pharmacological activity of the compound as COX inhibition, antioxidant activity and ion channel function or relevant to Huntington's disease (HD). Further evaluation of these genes is ongoing on cortical neurons

from a HD mutant mouse after *in vitro* treatment with BN82451B or vehicle. In this setting, BN82451B treatment was associated with an increase of neuronal viability. Altogether, these data demonstrate the pharmacological activity of BN82451B in the central nervous system and provide valuable information for biomarker exploration to improve the care of patients treated with BN82451B.

Disclosures: P.E. Chabrier: A. Employment/Salary (full or part-time):; Ipsen Innovation. **E. Aparicio:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **A. Bauchet:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **S. Rolland:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **A. Manon:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **P. Plas:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **M. Rocher:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **L. Naudin:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **Z. Zhang:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **V. Pierron:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **C. Benstaali:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ipsen Innovation. **L. Vignaux:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **M. Galcera:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **P. Roubert:** A. Employment/Salary (full or part-time):; Ipsen Innovation. **F. Schmidlin:** A. Employment/Salary (full or part-time):; Ipsen Innovation.

Poster

042. Huntington's Disease Clinical

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: 5R01NS055903-06

Title: Altered striatal resting state functional connectivity in children at risk for Huntington's disease

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Abstract: Background: Huntington's disease (HD) is an autosomal dominant disease caused by an expansion of the CAG repeats on the gene encoding for the huntingtin protein (*HTT*). The dysfunction of the striatum which shows selective vulnerability to mutant huntingtin (*mHTT*), is

known to underlie the characteristic HD symptom manifestation. Although the primary neuropathology is that of neurodegeneration of the striatum, the presence of *mHTT* throughout the life span may also affect normal maturational processes and circuit formation of the striatum that take place during development. Objective: In the current study, the effect of *mHTT* on striatal functional integrity was examined by evaluating resting-state functional magnetic resonance imaging (rs-fMRI) data in children (6-18 years of age) who are at risk for HD (no juvenile HD included). Method: Using seed-based analyses of the rs-fMRI data, the functional connectivity of the striatum in children tested as gene-expanded (n=26, CAG repeats ≥ 40) as a result of presymptomatic gene assessment (for research purposes only) were compared to that of 36 healthy children. Results: Compared to healthy peers, the gene-expanded children showed significantly weaker correlational strength between 1) the putamen and the motor cortex and 2) the ventral striatum and the anterior cingulate cortex. However, the gene-expanded children also exhibited significantly stronger functional coupling between 1) the caudate and the anterior prefrontal cortex, 2) the putamen and the superior frontal gyrus and 3) the ventral striatum and orbitofrontal cortex, when compared to healthy controls. Conclusions: The decreased functional synchronization between the striatum and associated frontal lobe areas in the gene-expanded children, indicate aberrant striatal functionality directly linked to *mHTT.* Interestingly, these decrements coincided with increased functional connectivity along the fronto-striatal circuitry which may reflect a potential compensatory mechanism. The results suggest that lifelong possession of *mHTT* could alter the striatum's intrinsic functional architecture, decades ahead of HD diagnosis. Importantly, understanding how the brain dynamically copes with such persistent effect of *mHTT* may help determine the most effective timing and target area to intervene prior to any irreversible structural degeneration.

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Poster

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Support: Ministerio de Economía y Competividad (Instituto de Salud Carlos III.Proyecto de Investigación en Salud).P14/00834

Ministerio de Ciencia e Innovación. PSI2011-23624

Title: Abnormal functional connectivity in Huntington's disease during a sequential motor task

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Abstract: Introduction: Huntington's disease (HD) is a neurodegenerative disorder that induces striatal and cortical neuronal dysfunction and loss which main symptom is motor impairment. Goals: to study whether this motor impairment is accompanied by changes in brain activity and functional connectivity. Methods: Eighteen early stage HD patients with a UHDRS motor score > 5 (10 men, mean age = 50.5 ± 10.08, mean TFC = 11.83 ± 1.17) and 19 controls (11 men, mean age = 49.21 ± 8.05) matched in age, gender and educational background underwent 3T structural and functional MRI scanning while they performed a sequential tapping task with their right or left hand in alternated blocks. Regional brain activation and functional connectivity were analyzed using mean number of tappings per block as a covariate of no interest to correct for performance differences between patients and controls. Results: Compared to rest blocks, active tapping activated the contralateral primary motor, premotor and supplementary motor areas, thalamus and cerebellum in both patients and controls. However, in the right hand condition, patients deactivated the right putamen, caudate, insula and premotor cortex to a greater extent than controls, which suggests that patients need a greater inhibition of the ipsilateral motor network in order to suppress the movement of the opposite hand. Bilateral primary motor cortex (M1), putamen and supplementary motor area (SMA) were selected as seeds region for a whole brain functional connectivity analysis. Both controls and patients showed negative connectivity between each of the seed regions and the motor network of the contralateral hemisphere. However, in the right hand condition, patients showed more negative connectivity of bilateral M1, primary somatosensory cortex (S1) and left secondary somatosensory cortex (S2) with the left putamen. Furthermore, the negative connectivity between the right M1 and the left putamen correlated with disease burden. In the left hand condition, right insula, M1 and premotor cortex were more connected (negatively) with the right putamen in patients compared to controls. Moreover, left S1 was more connected (negatively) with the right M1 in patients when compared to controls. Conclusions: These results indicate that HD patients need to inhibit the contralateral motor circuit of the moving hand to compensate for the hyperactivation of the dopaminergic system that underlies their motor symptoms.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Intramural Research Program

CHDI

Title: Equilibrative nucleoside transporter 1 (ENT1) as a biomarker of Huntington's disease

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Abstract: Preclinical, and epidemiological studies point to a role of adenosine in the pathogenesis of Huntington Disease (HD). We previously found that an adenosine A2A receptor (A2AR) antagonist did not produce locomotor activation in a transgenic HD rat model. The results were interpreted as due to a selective downregulation of striatal A2A receptors. But in the same animal model we also found a lack of locomotor activating effect of an A1R antagonist, while both A1R and A2AR agonists produced locomotor depression. The results therefore indicated a reduced adenosine tone. In fact, the striatal extracellular concentration of adenosine was significantly reduced in transgenic HD rats compared to controls. A decrease in striatal adenosine was also demonstrated in the present study in Q175 knock-in mouse. As a mechanistic explanation, we found that the striatal expression of the equilibrative nucleoside transporter 1 (ENT1) was significantly increased in Q175 mice. We then tested if a similar association could also be observed in humans. The gene expression profiles of 14 adenosine system genes, including ENT1, were analyzed in a large human cohort of 157 HD vs 155 control individuals (C). We first tested if any of these genes were up/down-regulated in HD relative to C using a differential expression (DE) analysis, and found that HD patients had a slightly higher but not significant transcript level of ENT1 in the frontal cortex than C. Nevertheless, the transcript level

of ENT1 was statistically higher in female, but not in male, HD patients compared with C of the same sex. We next inspected if the co-ordinate regulation of our genes of interest with other genes in the genome were disrupted (gained/lost) in the HD group relative to C using a differential coexpression (DC) analysis, a complementary approach to DE analysis utilized by several recent studies to identify disease-dysregulated genes. Based on a network of previously published DC relations identified at FDR 1% in this cohort, we found that some adenosine system genes were disrupted in hub-like DC patterns, similar to that observed for several other HD dysregulated genes. Specifically, ENT1 and also the ecto-nucleotidase CD73 were each differentially correlated with more than about 60 gene reporters at a statistically significant level, with mostly gain of correlations for ENT1 in HD patients relative to C.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CONACyT 369794

Title: Decisions relating to risk in patients with Huntington´s disease

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Abstract: Introduction: Huntington’s disease (HD) results in significant problems in judgement and decision making. However it is still unknown how early these capacities start to decline in these patients. **Objective:** Evaluate risky decision making in HD gene carriers with null or early clinical manifestations. **Participants:** 19 HD gene carriers with early evolution according to the scale of Total Functional Capacity UHDRS (TFD=11.7 ± 1.8); and 19 control volunteers

matched for sex, age and education. **Methods:** Subjects were evaluated using the Cambridge Gambling Task (CGT) of the CANTAB®. Participants saw ten boxes at the top of a screen, each of which is red or blue in some ratio. Under one of these boxes was a token, and participants had to guess whether the token was under the red or blue box. On a gambling trial, participants could select some proportion of their allotted points to bet on their judgment. Two bet conditions were applied, one ascending (possible bets start low and increase over time) and the other descending (bets start high and decrease over time). **Results:** The patient group bet a significant higher percentage of points in the descending condition (see Figure). Conclusion: HD gene carriers show a significant change in their decision making behavior even before revealing clinical changes. These changes from early stages of the disease suggest a judgment deterioration that could potentially put these patients in situations of risk; we suggest the development of strategies for individual care and judgment capacity adjusted for each patient.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS 38194

CHDI

Title: Regional stability of mutant htt mrna in post-rnai-induced degradation

Authors: *W. LIU, E. L. PFISTER, L. A. KENNINGTON, N. ARONIN;
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Abstract: Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder caused by expanded CAG repeats in first exon of HTT gene. The expanded CAG repeats encode a polyglutamine stretch near the N-terminus of the HTT protein. The CAG expansion is negatively correlated to the age of onset; particularly large expansions are linked to juvenile forms of the disease. There is no effective treatment available. Promising therapeutic strategies for HD include allele-selective and non-allele-selective RNAi techniques that target against the HTT mRNA. In either methods, RNAi induces a specific endo-nucleolytic cleavage of the target

HTT mRNA in the area complementary to the guide small RNA, and the resulting fragments are degraded by exo-nucleolytic process. We investigated the areal stability of HTT mRNA following the RNAi induced cleavage, especially the first exon that harbors the CAG repeats. YAC HD transgenic mice with 128 CAG repeats were injected with scAAV-mir-RNA-HTT-GFP designed against HTT targeting the middle part of transgenic HTT (exon 48). Transgenic HTT mRNA levels were estimated after two weeks, using primer-probe combinations covering different parts along the HTT mRNA. Our results show that at six sites examined, transgenic mutant HTT reduced to 63.9% to 71.2% in striatum injected with scAAV9-U6-mir-HTT-GFP. However, there was no significant difference between sites. Our results demonstrate that expanded CAG repeats in mutant HTT did not stop or slowdown the mRNA degradation following the RNAi-induced cleavage.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Indian Council of Medical Research, New Delhi, India

Title: Utility of genetic testings for clinically suspected Huntington’s disease and Spinocerebellar Ataxias from Movement Disorder clinic in India

Authors: *S. D. VENKATESH;
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Abstract: Objective: To study the utility of genetic testing for Huntington’s disease(HD) and spinocerebellar ataxia (SCA1, SCA2, SCA3 and SCA12) in referred from a Movement Disorder clinic in a tertiary care hospital in India. Background: HD and SCA are neurodegenerative conditions that haveworldwide distribution.The prevalence of HD varies from 0.6-10 per million in Asian to European populations.SCA3 is the most frequent mutation detected worldwide followed by SCA2 and SCA1. SCA12 is found prominently in specific Indian ethnic group. Only limited data is available on occurrence of HD and SCA in Indian population. Methodology: Subjects clinically suspected for HD (N=175) and SCA (N=844) were recruited from outpatient and inpatient referrals to Genetic Counselling and Testing Centre (GCAT) at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India after obtaining

informed consent with detailed explanation and genetic counselling. The genomic DNA was extracted from whole blood by salting out method. Polymerase chain reaction (PCR) was setup using appropriate primers to identify the HD, SCA1, SCA2, SCA3 and SCA12 mutation. The expanded CAG repeats were identified by high resolution agarose gel electrophoresis of the amplified products, followed by fragment analysis forrepeat sizing using ABI 3500xL and Gene Mapper v3.5 respectively. Result: Of the 175 subjects clinically suspected for HD, pathological expansion was confirmed in 120 (68.5%) samples (symptomatic, n=106, and pre-symptomatic, n=14. Among the 844 subjects clinically suspected for SCA, 246(28%) subjects had identifiable SCA mutations.SCA1 was most common [n=100 (12%)] followed by SCA2 [n=98 (11%)],SCA3 [n=40 (5%)] and SCA12 [n=8 (1%)]. Most of the confirmed HD cases were of southern Indian origin. Genetically confirmed SCA1, SCA2 and SCA3 cases were predominantly from southern India, whereas SCA12 was predominantly from northern India. Conclusion: Our study provides a large sample data on the occurrence of HD and SCA (SCA1, SCA2, SCA3 and SCA12) mutations from the patients recruited in GCAT clinic, NIMHANS, India. Pre-test and post-test counseling, as well as long term follow-up and care needs for this cohort and their families needs to be developed. This study is informative guide for prioritizing health care policies towards movement disorders in India.

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Poster

042. Huntington's Disease Clinical

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ANR-10-LABX-73

Title: An original approach for personalized parcellation of macaque MR brain images: application to caudate volume estimation in a model of Huntington’s disease

Authors: *Y. BALBASTRE[1,2], M. E. VANDENBERGHE[1,2], J. FLAMENT[1,3], A.-S. HÉRARD[1,2], P. GIPCHTEIN[1,2], S. WILLIAMS[1,2], N. SOUEDET[1,2], M. GUILLERMIER[1,2], A. BUGI[5], A. PERRIER[6,4], R. ARON-BADIN[1,2], P. HANTRAYE[1,2], J.-F. MANGIN[7], T. DELZESCAUX[1,2];
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France; [3]Inserm, Fontenay-aux-Roses, France; [4]U861, Inserm, Evry, France; [5]CECS, [6]UEVE U861, AFM - I-STEM, Evry, France; [7]CEA / NeuroSpin, Gif-sur-Yvette, France

Abstract: In the last couple of decades, non-human primates (NHP) played major roles in the development of treatments for Parkinson's and Huntington's diseases. Because of their high genetic similarity with humans, they bridge the gap between rodents and humans for therapy development and evaluation. However, translational research success from NHP to patients requires accurate phenotyping of the models. Magnetic resonance imaging (MRI) combined with automated segmentation methods offers the unique opportunity to assess brain morphological changes caused by neuronal degeneration in patients (volume, cortical thickness, sulci morphology). While a longitudinal follow-up of such parameters is essential in NHP models, few robust algorithms (Ballanger et al. 2013) are available because NHP brains are small and high magnetic fields used in preclinical imaging produce different contrasts. To overcome these obstacles, we propose an automated method (www.brainvisa.info) that merges structural information from a macaque brain probabilistic digital atlas (Rohlfing et al. 2012) and local information from MR signal We studied a common NHP model of striatum-related pathologies. Male macaca fascicularis injected with quinolinic acid (QA) in the striatum (n = 8) were compared to healthy ones (n = 13). All subjects were submitted to T2-weighted MRI at 7 Tesla (Varian). We developed an algorithm enabling accurate delineation of various neuroanatomical regions of interest (ROIs) from individual MR images. The atlas was warped on each MRI and used as prior to estimate ROI intensity profiles used to correct the atlas probabilities and match at best each individual brain anatomy. Images were finally segmented into 23 ROIs, for which volumes were computed. As a control, caudate nuclei from 7 animals were manually segmented on MR images. Our method was successfully applied to all healthy subjects and 75% of QA subjects. Delineated caudate volumes showed good correlation with control segmentations (our method: r^2=0.89; atlas registration: r^2=0.08). As expected, healthy and lesioned subjects were easily identified based on caudate volumes normalized for intracranial volume (T-test; left: p < 10-7; right: p < 10-4; Bonferroni correction). This method could be of major interest to characterize the shape and size of neuroanatomical ROIs longitudinally. This approach looks like a good complement to voxel-based analyses in highly variable NHP models and provides a valuable brain parcellation to analyze functional images. Some applications could be stereotaxy quality control and biomarker quantitation for *in vivo* evaluation of models and therapies.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Oberlin College Office of Foundation, Government, and Corporate Grants

Title: Acute chlorpyrifos exposure induces oxidative stress and mitochondrial dysfunction in a striatal cell model of Huntington's disease

Authors: *G. A. DOMINAH[1], G. F. KWAKYE[2];
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Abstract: In spite of the genetic cause of Huntington's disease (HD), emerging evidence suggests that potential exposure to environmental factors may contribute to the variability in the age of onset, progression, and severity of the disorder. However, the identity of the environmental risk factor is currently unknown. Recognizing some of the shared similarities in the pathophysiological mechanisms in HD and pesticide neurotoxicity such as oxidative stress and mitochondrial dysfunction, we hypothesized that the commonly used agrochemical, chlorpyrifos (CPF) would exhibit a disease-toxicant interaction and reveal the influence of CPF in HD neuropathology. Following a 48-hour exposure to the metabolites CPO and TCP, we report no significant dose and genotypic differences in cell survival. However, mutant $STHdh^{Q111/Q111}$ cells show increased susceptibility to CPF neurotoxicity compared to wild-type $STHdh^{Q7/Q7}$ cells. In addition, mutant $STHdh^{Q11/Q111}$ cells exhibit increased production of reactive oxygen species and diminished glutathione levels in a dose-dependent manner compared to wild-type $STHdh^{Q7/Q7}$. We examined the possibility of the antioxidant N-acetylcysteine (NAC) treatments to ameliorate the genotypic difference in cell survival. Pre- and simultaneous, but not post treatments to striatal cells with NAC was sufficient to decrease CPF induced toxicity and abolish the genotypic difference in the striatal HD cells. Moreover, we report decreased net energy production as well as decreased mitochondrial membrane potential in mutant $STHdh^{Q111/Q111}$ cells compared to wild-type $STHdh^{Q7/Q7}$ cells following CPF exposure. In addition, heme-oxygenase 1 (HO-1) protein expression levels, a biomarker for oxidative stress, increase in both $STHdh^{Q7/Q7}$ and $STHdh^{Q111/Q111}$ cells suggesting that CPF causes neurotoxicity via oxidative stress pathways to induce striatal neuron loss even without the influence of mutant Htt protein.

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Poster

042. Huntington's Disease Clinical

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Swedish Brain Foundation

Title: Pridopidine selectively occupies sigma-1 rather than dopamine D2 receptors at low, behaviorally active doses

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Abstract: Dopamine stabilizers have stimulatory actions under low dopamine tone and inhibitory actions under high dopamine tone without eliciting catalepsy. These compounds are dopamine D2 receptor (D2R) antagonists or weak partial agonists, and may have pro-mnemonic and neuroprotective effects. The mechanism underlying their stimulatory and neuroprotective actions is unknown but could involve sigma-1R binding. The present study examined sigma-1R and D2R occupancy by the dopamine stabilizer pridopidine (ACR16) at behaviorally relevant doses in living rats. Rats were administered 3 or 15 mg/kg pridopidine, or saline, before injection of the radiotracer 11C-SA4503 (sigma-1R) or 11C-raclopride (D2R). Some animals received 60 mg/kg pridopidine and were only scanned with 11C-raclopride. Cerebral 11C-SA4503 binding was quantified using metabolite-corrected plasma input data and distribution volume (VT) calculated by Logan graphical analysis. 11C-raclopride binding was quantified using striatum-to-cerebellum ratios and binding potentials calculated with a simplified reference tissue model (SRTM). Cunningham-Lassen plots indicated sigma-1R occupancies of 57 ± 2% and 85 ± 2%

after pretreatment of animals with 3 and 15 mg/kg pridopidine. A significant (44-66%, as determined using SRTM and striatum-to-cerebellum ratio, respectively) reduction of 11C-raclopride binding was only observed at 60 mg/kg pridopidine. At doses shown to elicit neurochemical and behavioral effects, pridopidine occupied a large fraction of sigma-1Rs and a negligible fraction of D2Rs. Significant D2R occupancy was only observed at a dose 20-fold higher than was required for sigma-1R occupancy. The characteristics of dopamine stabilizers may result from the combination of high sigma-1R and low D2R affinity.

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Poster

042. Huntington's Disease Clinical

Location: Hall A

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant PN2EY016525

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Title: Cerebrospinal fluid from HD subjects seeds aggregation of mutant huntingtin

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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disease that results in progressive motor, cognitive and psychiatric impairment and ultimately death. The disease is caused by an abnormal CAG trinucleotide repeat expansion within exon 1 of the HD gene that produces a mutant Huntingtin protein (mHTT) containing an expanded polyglutamine (poly(Q)n, n≥ 36) tract. The length of the expansion has an inverse relationship to age-of-onset

of clinical motor symptoms, but onset of symptoms in mutant Huntingtin gene-carrying individuals remains unpredictable. We report that cerebrospinal fluid from BACHD transgenic rats and from human Huntington's subjects effectively seeds mutant Huntingtin aggregation in a cell model and its cell lysate. Our studies demonstrate that seeding is mutant Huntingtin template-specific and may reflect an underlying prion-like protein propagation mechanism. Light and cryo-electron microscopy show that synthetic seeds nucleate and enhance mutant Huntingtin aggregation. This seeding assay distinguishes blinded living Huntington's subjects from healthy and non-Huntington dementia controls without overlap. Seeding measures from expansion-gene-positive subjects without clinical symptoms range from HD to control values. Ultimately this seeding property in Huntington's patient cerebrospinal fluid may form the basis of a molecular biomarker assay to monitor HD and evaluate therapies that target mutant Huntingtin protein.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 43.01/C70

Topic: C.10. Trauma

Support: Army CCCRP: H_005_2012_WRAIR

Title: Differential profiles of molecular pathology following repeat concussion in an animal model of projectile concussive impact (PCI) injury

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Abstract: Closed-head concussive injury is one of the most common causes of traumatic brain injury (TBI) and represents a significant public health concern. While neurologic dysfunction from a single concussion may be limited, multiple concussions can result in cumulative damage and increased risk for long-term morbidity. However, there is very limited understanding of the clinical intervals between diagnosed injuries and emergence of latent pathologies. In the current study, we evaluated changes in key TBI markers after both single and repeated concussions using the WRAIR projectile concussion impact (PCI) model in rodents. Importantly, PCI caused

a delay in righting reflex indicative of mTBI in our model. Following PCI injury, cortex (CX) and hippocampus (HC) tissue lysates were evaluated as select regions of interest and compared to sham controls. Single PCI was evaluated at 4h, 24h, 72h and 7 days post injury; but no significant changes in APP, GFAP or tau were detected in the CX or HC. Repeat PCI injuries (4 injuries) were conducted at 1h, 24 hour or 7 day inter-injury intervals. Experimental endpoints included 3 days after the last injury (all injury cohorts) as well as 24h and 7 days post-PCI (1h inter-injury intervals only). Following 4 PCI injuries spaced 1h apart, significant increases in tau (33%) and phosphorylated tau (28%) were detected in the HC at 3 days post-injury that were normalized by 7 days post-injury. No changes were found in the CX. No changes were found in APP or APP beta cleavage at 3 or 7 days. Increased GFAP (138%) was seen in CX but not HC. Following 4 PCI injuries spaced 24h apart, no changes were seen in tau phosphorylation, APP or APP beta cleavage at 3 days post injury. However increased GFAP was detected in both CX (207%) and HC (44%) at 3 days post injury. No significant changes were detected in any of these markers following 4 PCI injuries spaced 7 days apart. Notably, the 24h interval between concussions produced substantially greater GFAP upregulation vs. the 1h interval, an affect that was observed in both CX and HC regions. Overall, these results indicate that tau and tau phosphorylation are more affected following rapid repeat injuries; whereas GFAP changes are more prevalent with longer inter-injury intervals and thus may prove more valuable for evaluating therapeutic interventions.

Disclosures: **C.M. Cartagena:** None. **A.M. Boutte:** None. **H. Hwang:** None. **D. Johnson:** None. **F.C. Tortella:** None. **D.A. Shear:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

Location: Hall A

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Topic: C.10. Trauma

Support: NIH Grant R01HD055813

Title: Effects of diffuse axonal injury (DAI) in the retinal-thalamic pathway upon the synaptic input and intrinsic properties of relay cells in the dorsal lateral geniculate nucleus (dLGN)

Authors: *C. W. JURGENS, V. C. PATEL, T. E. KRAHE, J. T. POVLISHOCK;
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Abstract: Visual deficits are a common occurrence in many types of traumatic brain injury (TBI), with some deficits resolving while others persist. Our lab has previously shown scattered axonal disconnection and dieback of axons in the mouse optic nerve following the central fluid percussion injury (cFPI) model of DAI. Here we utilized the mouse visual system to assess the functional consequences of DAI on visual circuits and their recovery. It is widely accepted that axonal deafferentation can induce synaptic plasticity and rearrangement in the cortex leading to at least some recovery of function. However, little is known about the contribution of the thalamus in recovery following DAI. To examine this at the synaptic level, we utilized whole cell patch clamp recordings from the dorsal lateral geniculate nucleus (dLGN) to assess the functional implications in the primary visual thalamus at 10 and 20 days post cFPI and its associated DAI. Slices containing an intact optic tract were used to determine differences in connectivity, EPSC characteristics and cell membrane properties between injured and sham mice. Our findings demonstrated a decrease in the number of inputs to each cell with little change of the remaining EPSC dynamics. Importantly, this decreased input persisted over the 20 day post injury period evaluated in this study. While the overall functional implications of these findings remain to be assessed, these persistent alterations in dLGN synaptic input would not suggest an adaptive response at the thalamic level. Although more work is required, these studies support the premise that the visual system may serve as an excellent model for further understanding the underlying interaction and compensation of circuitry pathways in some of the recovery associated with DAI.

Disclosures: C.W. Jurgens: None. **V.C. Patel:** None. **T.E. Krahe:** None. **J.T. Povlishock:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

Location: Hall A

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Topic: C.10. Trauma

Support: HD055813

Title: Structural studies of plasticity in dorsal lateral geniculate nucleus following TBI

Authors: *V. C. PATEL, T. E. KRAHE, J. T. POVLISHOCK;
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Abstract: Diffuse axonal injury (DAI) or traumatic axonal injury (TAI) is a consistent feature of most forms of traumatic brain injury (TBI) and is associated with most of its morbidity. Little is known however regarding its downstream consequences. Previous publications from our lab demonstrate that even mild TBI injury can induce TAI within the optic nerve. The result is diffuse and scattered pattern of axonal loss despite retention of a large fraction of optic nerve axons. In the current study we seek to evaluate if the remaining intact axons demonstrate adaptive or maladaptive plastic response. To do so, we utilized epifluorescent, and electron microscopy to study intact retinal ganglion axonal terminals and their potential rearrangement within dorsal lateral geniculate nucleus (dLGN) over a 20 day post injury period. Using 9 to 12 week old C57B6J mice, we performed central fluid percussion injury (~1.4 ATM), with the use of appropriate shams. On post-injury days 1, 7, and 17, CTB conjugated Alexa dyes are injected into the vitreous cavity of both eyes using separate colors for each eye. Seventy-two hours following eye injections, animals are perfused and coronal slices prepared. Projections from either eye were mapped using epifluorescent images with thresholded analysis to compare area of dLGN covered by ipsilateral and contralateral projections. Fluorescent signal intensity was compared between experimental groups within the monocular zone of dLGN using ImageJ on raw images. To confirm these fluorescent findings at the EM level electron dense conversion of CTB Alexa 488 was performed in additional animals at corresponding time points. Through this approach we found within dorsal LGN, scattered loss of axonal terminal input contiguous with retinal ganglion cells. We observed ~40% loss of ipsilateral retinal projections with ~20% loss of contralateral projections following injury. This distribution of terminal loss remains unchanged over the 20 day post-injury period. No evidence for synaptic rearrangement of overlap between ipsilateral and contralateral projections in dLGN was found. We believe there is limited to no anatomic adaptive plasticity of retinal ganglion axon terminals within adult mouse dLGN following a mild fluid percussion injury associated with diffuse axonal injury.

Disclosures: **V.C. Patel:** None. **T.E. Krahe:** None. **J.T. Povlishock:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Support: Neilsen Foundation #82776

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DOD USAMRMC CDMRP FSECSTS EMS# 12339079

Title: Acute traumatic injury to human and porcine astrocytes associates with new biomarker release and proteolytic cleavage

Authors: *I. B. WANNER[1], J. HALFORD[1], K. ITAMURA[1], J. LEVINE[1], S. SHEN[2], J. A. LOO[2], T. C. GLENN[3], S. MONDELLO[4], D. W. DIETRICH[5], K. BARAZANJI[6], A. GIGLIOTTI[7], A. W. MAYER[7];
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Abstract: A proteomic study after *in vitro* trauma yielded a list of fast trauma-released proteins, the mouse traumatome (Levine J et al., submitted). Cerebrospinal fluid (CSF) from traumatic brain injury (TBI) patients carried a strong astroglial signature determined by mass spectrometry. A new neurotrauma marker panel was identified from the overlap between traumatome and TBI CSF proteome by selecting astrocyte enriched proteins not found in healthy blood. Aldolase C (ALDOC), brain lipid binding protein (BLBP) and, for study rigor, glial fibrillary acidic protein (GFAP) were chosen from this panel. Their levels were significantly elevated in a cohort of TBI patients' CSF and blood samples versus controls and all displayed proteolytic breakdown products (BDPs). ALDOC and BLBP were faster released and had a longer detection window than GFAP. To further characterize their pathobiology, we stretch-injured *in vitro* matured human fetal astrocytes (Wanner I, 2012). ALDOC and BLBP were significantly released within 30 mins post-injury and were associated with trauma-inflicted membrane permeability, mechanoporation. GFAP 25-19 kDa lower BDPs were significantly released by 48 hours after stretching and were associated with cell death. Pharmacological inhibition of calpains and caspases suggest that both contributed to trauma induced proteolytic marker cleavage. Human traumatized astrocytes had process fragmentation and beading as well as GFAP filament disassembly and altered antigenicity. Fast released markers were depleted from mechanoporated cells acutely after injury. These changes were also found *in vivo*, in preliminary acute spinal cord injury (SCI) studies in the yucatan swine. Swine CSF levels for the three astroglial markers were elevated 20 mins to 3 days after mild-to-moderate contusion SCI and were associated with astroglial histopathology. Porcine astrocytes share complexity of human astrocytes including interlaminar processes. Astrocyte beading and process fragmentation were seen in white and gray matter near the lesion acutely post-injury. ALDOC and BLBP levels were reduced in lesion-near, injured astrocytes compared to least affected astrocytes in lesion-far areas. Process fragmentation was measured using new semi-automated image analysis to quantitate diffuse astrocyte injury. Astrocyte soma swelling is now quantifiable using ALDOC as feasible

cytoplasmic astrocyte marker. In conclusion, we provide new trauma-released astroglial injury markers that are released by mechanoporation and cell death, are cleaved by trauma-activated calpains and caspases and can be valuable as future diagnostic and predictive neurotrauma biomarkers.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Support: P30 GM103340

Title: Neurotrauma in young mice induces changes in neuroprotectin D1 (NPD1) and other lipid mediators in brain and serum

Authors: *J. L. ROSSI[1], E. J. KNOTT[1], C. L. DAVIDSON[1], T. TODD[1], B. JUN[1], L. H. HOLLIER[2], N. G. BAZAN[1];
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Abstract: Introduction: Traumatic injury to the skull and dura affects multiple brain cells perturbing neurotransmission, ion channels and early neural circuit establishment. Lipid signaling has been explored in TBI; however, the significance of the newly discovered neuroprotective docosanoids is not understood under these conditions. Here we examined early responses in young mice following closed head TBI aiming to understand the significance of the expression of protective lipids as a function of early age, time and severity of injury. **Methods:** PND21, PND24 and PND35 mice were anesthetized with avertin, mechanically ventilated, physiologically regulated, and subjected to a lateral closed-skull injury model with impact depth of 2mm- moderate, or 2.25 mm- severe injury (bregma level - 0.10mm). Mice sacrificed timepoints were T<2m (minutes), 30m, 1h (hour) and 4h and 24h. Lipids were extracted by solid phase and run by LC-MS/MS. **Results:** NPD1 shows significant elevation in the serum of PND35 mice at 24h after severe closed head traumatic brain injury (SCHT) [sham (s)-8.838 vs. severe-20.50] and when deuterium labeled DHA was injected 7d prior to impact an increased

level of D5-NPD1 in the serum was observed over sham operated animals [s-69.77 vs. severe-295.8]. This also matched with the endogenous LC-MS profile of enhanced NPD1. NPD1 also displayed a trend of enhancement at 1h in PND21 brains between moderate and severe impact depths [moderate-6.5 vs. severe-14.17]. Within two minutes of SCHT we found a selective, rapid and transient accumulation of 15- HETE in both PND24- [sham (s) 4.833 vs severe 15.5)] and PND21-[s 5.16 vs. severe 13.17]. PND24 mice also showed a rapid increase in PDG2 and PGE2 in both, with significance between sham and severe injuries at T< 2m for both [s-3.88 vs. severe 14.33] as well as in PND21 mice both PDG2 and PGE2 show significance at T< 2m in both depths of injury, sham vs. moderate (4.33 vs. 11.83) and sham vs. severe (4.33 vs. 12.33). HETE 12 shows significance in PND21 mice between shams vs. moderate injury at 1h (5.667 vs. 14). 12-HETE also trends slowly to elevation in the SCHT through 4h in PND24. **Conclusion:** This is an initial study to explore lipid mediators engaged in the neuroinflammatory response in TBI of young mice. The evidence of NPD1 in the serum is novel and provides potential usefulness as a marker. The rapid and transient upregulation of 15-HETE, PDG2 and PGE2 requires further investigation.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

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Title: Newly identified role for Arylsulfatase B in the upregulation of chondroitin-4-sulfate glycosaminoglycans after brain injury

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Abstract: Chondroitin-4-sulfate glycosaminoglycans (C4S-GAGs) are upregulated after brain injury and inhibit effective axonal regeneration, therefore contributing to permanent brain damage and impaired cognitive functions. Chondroitin 4-sulfotransferase 1 (C4ST1, also called CHST11), the last enzyme in the biosynthesis of C4S, has been reported to be upregulated in animal models of traumatic brain injury, indicating that injury triggers C4S biosynthesis. In this study we explored another mechanism by which C4S is upregulated after injury, i.e. through the inhibition of the activity of arylsulfatase B (ARSB), the enzyme that removes sulfate groups from C4S triggering the degradation of C4S-GAGs. Following penetrating ballistic-like brain injury, C4S-GAG levels increased in association with ARSB activity inhibition and C4ST1 mRNA expression and sulfotransferase activity upregulation at the ipsilateral site of injury, but were unaffected at the contralateral site or in sham controls indicating contributions from both increased production and reduced degradation to the accumulation of C4S following trauma. Reactive astrocytes are responsible for the dramatic increase in the levels of extracellular CS-GAGs at the lesion site. It has been reported that the phenotype of reactive astrocytes is not homogeneous and depends on the type of insult astrocytes are responding to. In the model of PBBI used in this study, the brain undergoes mechanical damage, which rapidly triggers an inflammatory response. In order to dissect the relative contribution to C4S-GAGs upregulation of mechanically-injured and inflammatory factors-exposed reactive astrocytes, we used two well-established *in vitro* models of reactive astrocytes: the scratch-would model and the transforming growth factor β1 (TGFβ1)-induced *in vitro* astrogliosis model respectively. The total levels of C4S-GAGs was increased in both models of reactive astrocyte; however, while scratch injury inhibited ARSB activity without affecting C4ST1 expression or sulfotransferase activity, TGF-β1 exposure increased C4ST1 expression and sulfotransferase activity with no effect on ARSB. Hence, inhibition of ARSB activity represents a newly identified mechanism by which glial scar C4S-GAGs are upregulated as a result of the activation of astrocytes selectively by mechanic injury. Based on these results, recombinant ARSB therapy, already in use to treat mucopolysaccharidosis VI (a genetic defect in the ARSB gene leading to deficiency in the ARSB enzyme) should be considered to reinstate plasticity after brain injury.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Support: NJ Commission on Brain Injury Researc

American Academy of Neurology

Title: Ephrin signaling in axon regeneration as a target for the treatment of traumatic brain injury

Authors: A. GIARRATANA[1], A. PALMIERI[1], S. TENG[1], J. PARK[1], R. ZHOU[2], J. ALDER[1], *S. THAKKER-VARIA[1];
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Abstract: Molecules of the Eph/Eprhin family are repulsive to process outgrowth and recent studies suggest that blocking Eph function or expression promotes recovery following spinal cord injury. However, the effect of inhibition of Eph receptors in traumatic brain injury (TBI) has yet to be investigated. We have shown that the EphA6 receptor is expressed in the adult brain and is upregulated in the hippocampus following the lateral fluid percussion (LFP) model of TBI. In EphA6 mutant mice, neurons grow abnormally exuberant processes, supporting the notion that the EphA6 receptor functions to restrict growth of axons and dendrites. There is high expression of the ligand for this receptor, Ephrin-A5, in the hippocampus as well as in motor and sensory cortex of adult mice and we have shown that Ephrin-A5 is also inhibitory to process outgrowth *in vitro*. In the current study, we have used soluble Eph and Ephrin-Fc fusion proteins infused into the adult cortex and hippocampus to examine regeneration and behavioral recovery following LFP injury in adult male mice. The antagonist EphA6-Fc, the agonist EphrinA5-Fc or control IgG were administered to cortex of the wildtype mice via mini-osmotic pumps for one week following LFP. Sham mice were used as control. EphA6-Fc treated mice showed significantly less cell death as indicated by activated caspase-3 positive cells and fewer degenerating neurons as evidenced by Fluorojade-C labeled cells following LFP compared to IgG treated animals. There are also fewer GFAP positive cells in the EphA6-Fc treated mice suggesting a reduction in glial scar formation. The mice treated with EphA6-Fc also had a longer latency to fall on the rotarod 21 days after injury indicating improved motor function compared to control mice. Conversely, in mice treated with the EphrinA5-Fc, which acts as an agonist to the EphA6 receptor there was increased apoptosis and a trend to exacerbated motor function relative to control. The effect of EphrinA5-Fc on cell death, degeneration and glial scar formation are currently under investigation. We are also using EphA6 mutant mice crossed to transgenic Thy1-YFP mice to visualize axons and determine if the absence of EphA6 signaling enhances regeneration. Together these studies will yield insights into the role of Eph/Ephrin signaling in injury and demonstrate the therapeutic potential of Fc fusion proteins for the treatment of TBI.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

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Title: Diffusion MR imaging reveals abnormalities in the corpus callosum after single TBI versus overlying cortex after repetitive TBI

Authors: ***F. YU**[1,2], D. SHUKLA[2], R. ARMSTRONG[2,1], B. DARDZINSKI[2], R. SELWYN[3,2]; [1]Dept. of APG, [2]Ctr. for Neurosci. and Regenerative Med., Uniformed Service Univ., Bethesda, MD; [3]Radiology, Univ. of New Mexico, Albuquerque, NM

Abstract: Non-invasive detection of brain abnormalities from single and repetitive mild traumatic brain injury (TBI) is important for evaluation of the acute through chronic effects of impact-acceleration head injuries. Magnetic resonance imaging (MRI) is beginning to reveal findings in mild TBI patients that are not detected with conventional imaging. This MRI study used diffusion tensor imaging (DTI) to evaluate longitudinal changes in both the corpus callosum and the overlying cortical gray matter after single and repetitive impact injuries in adult male C57BL/6 mice. For single TBI (sTBI), mice had a scalp incision to expose the skull and received a stereotaxically controlled impact at bregma (3 mm tip; 1.5 mm depth; 4.0 m/sec; 100 msec dwell time). For repetitive TBI (rTBI), mice received a milder impact (1.0 mm depth; 4.0 m/sec; 200 msec dwell time) onto the scalp over bregma once each day for 5 days. Sham mice were run in parallel but without impact. T2-weighted MRI and DTI scans were performed at baseline and at 3 dys, 6 dys, and 6 wks post-TBI/sham. Fractional anisotropy (FA) values were significantly decreased in the corpus callosum after sTBI, but not rTBI. The FA decrease corresponded with a decrease of axial diffusivitiy (AD) and increase in radial diffusivity (RD), although neither AD or RD changes reached significance. Histological analysis of the corpus callosum showed significant axon damage, astrogliosis and microglial activation after sTBI; after rTBI, axon damage, astrogliosis and microglial activation was significantly increased compared to sham mice but was milder than after sTBI. Significant demyelination was found in the corpus callosum after sTBI but not following rTBI. In contrast to the corpus callosum, in cortical regions DTI showed reduced AD in the rTBI mice, but not in sTBI mice. FA and RD were not significantly

changed in the cortex after rTBI. These differential DTI findings indicate that rTBI can result in milder damage in the corpus callosum while having more significant changes in the overlying cortex following repetitive impacts that are each milder than the impact used in the sTBI model. The DTI evidence of cortical abnormalities in the rTBI mice is supported by behavioral deficits in social interaction at 3 wks after rTBI, but not sTBI. Respective DTI changes, in the corpus callosum after sTBI and in the cortex after rTBI, persisted through 6 wks post-TBI. These findings demonstrate that evaluation of mild rTBI should include region-of-interest analysis in the cerebral cortex and could be missed with widely used approaches for DTI analysis that are confined to white matter tracts.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

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NIH Grant CA158402

Title: The role of par1 in regulating neuroinflammation following traumatic brain injury

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Abstract: Millions of Americans are living with disabilities caused by traumatic brain injuries (TBI), making it a serious public health concern. Despite the overwhelming incidence of TBI, there is a lack of treatment options for those injured. A major obstacle of TBI recovery is the secondary neuronal damage caused by a prolonged neuroinflammatory response. The chief cells mounting the neuroinflammatory response are microglia, the brain's resident immune cell. However, mechanisms underlying microglial activation are still unclear. Following TBI, microglia transform from a highly ramified state to an activated state, where they lose most of their spatial asymmetry and become rounded. This raises the exciting possibility that proteins

regulating cellular polarity/asymmetry are involved in the microglia activation process. The partitioning-defective (Par) proteins are central regulators of polarity establishment in many different cellular contexts including embryogenesis, directional motility, epithelial morphogenesis, axon specification and dendritic spine formation. Here, we show that one of the polarity regulators, the Ser/Thr kinase Par1, play an important role in microglia activation following TBI. We show that Par1b knockout (KO) mice exhibit heightened basal levels of microglia activation, indicated by their altered morphology. Following controlled cortical impact (CCI) injuries, Par1b KO mice show excessive microglia activation that spreads more distally from the injury site than wild-type (WT) controls. Interestingly, even sham-operated KO mice show significant microglia activation, suggesting that the microglia in the Par1b KO mice are hypersensitized to insults. Further, knockdown of Par1 in primary microglia cultures resulted in morphological changes from a ramified to an amoeboid shape, which is reminiscent of activated microglia. By contrast, overexpression of Par1 blocked ATP-induced morphological changes in microglia. Moreover, we found that microglia depleted of Par1 phagocytize significantly more damaged neuronal particles than controls. Taken together, we propose that decreased Par1 activity and expression leads to increased neuroinflammation and worsens TBI outcomes.

Disclosures: V.L. DiBona: None. **K. Krause:** None. **L. Bernard:** None. **Q. Wu:** None. **D. Crockett:** None. **H. Zhang:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 43.10/C79

Topic: C.10. Trauma

Support: Academy of Finland

Finnish Epilepsy Research Foundation

Title: Magnetic resonance spectroscopy (MRS) of post-traumatic epileptogenesis

Authors: *A. YASMIN, O. GRÖHN, A. PITKÄNEN, R. IMMONEN;
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Abstract: Traumatic brain injury (TBI) leads to post-traumatic epilepsy (PTE) in up to 53% of patients. In many cases, epileptogenic focus develops to the perilesional cortex. Here we hypothesized that TBI induces a metabolic fingerprint to the perilesional cortex that predicts

epileptogenesis. To address the hypothesis we used magnetic resonance spectroscopy (1H-MRS) to reveal metabolic alterations in the perilesional cortex *in vivo*. Methods: Fifteen *in vivo* detectable neurochemicals were analyzed in the perilesional cortex in lateral fluid-percussion injury (LFPI) rat model of TBI. Adult, male Spraque-Dawley rats (n=20 TBI, 10 sham) were imaged at 1, 3, and 6 months post-TBI. 1H-MRS was carried out at 9.4 Tesla high field magnet. Spectra were obtained from single perilesional voxel (1*3*5mm) by PRESS (TE 11ms, TR 2500ms, 320/640 averages for sham/TBI). Spectra were analyzed by LC model and only metabolites with SD%≤ 20 were included. Metabolite concentrations were normalized to creatine and phosphocreatine (Cr+PCr) peak to account for the tissue atrophy. In the end, rats were monitored for 4 wk with 24/7 video-EEG to detect epileptiform activity. Results: 6 out of 15 parameters showed changes at some follow-up point. Myo-inositol was increased up to 77% ($p<0.01$) at 1 month, 35 %($p<0.01$) at 3 months, and 21% ($p<0.01$) at 6 months post-TBI as compared to corresponding controls. There was partial recovery of elevated Myo-inositol levels among the injured animals ($p<0.01$, between 1 month and 6 months post injury). At 6 months post-TBI, NAA was increased by 14% ($p<0.05$) as compared to controls, and by 16 % ($p<0.01$) as compared to 1 month post injury animals. Also perilesional NAA+NAAG levels in trauma animals elevated 14% over time ($p<0.01$, 1 month vs 6 months) despite the progressive atrophy of the primary lesion. Glutathione (GSH) was increased by 17% ($p<0.05$) in 1 month post-TBI. Glycerophosphocholine (GPC) alone and with phosphocholine (GPC+PCh) both showed an increase over time in the TBI group (GPC 12%, GPC+PCh 11%, $p<0.05$ from 1 to 3 months). In further analysis the MRS findings will be correlated with the seizure susceptibility in EEG. Conclusions: We found markedly increased myo-inositol levels in the perilesional cortex, indicating gliosis. The elevated glutathione may reflect potential on-going oxidative stress. Phosphocholines are linked to membrane turnover. Whether any of these metabolic anomalies could identify the epileptogenic animals is yet to be examined.

Disclosures: **A. Yasmin:** None. **O. Gröhn:** None. **A. Pitkänen:** None. **R. Immonen:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Support: Funded by the DoD in the Center for Neuroscience and Regenerative Medicine (CNRM).

Title: Intracerebroventricular transplantation of adult neural stem cells after traumatic brain injury: a proof-of-concept for activation of host neural stem cells in the subventricular zone

Authors: *G. SULLIVAN, R. C. ARMSTRONG;
APG, USUHS, Bethesda, MD

Abstract: Transplantation of neural stem cells (NSCs) may promote brain repair by replacing lost cells and by interacting with the host tissue to modulate the immune response and stimulate endogenous regenerative capacity. Determining an effective NSC delivery route in the CNS is particularly challenging for traumatic brain injury (TBI) that involves diffuse rather than focal lesions. In clinical management of TBI, ventriculostomy is often performed in patients with acute TBI after failure to control intracranial pressure by other means. Ventriculostomy may provide a route of access to the lateral ventricle for therapeutic delivery of stem cells. The current studies provide a proof-of-concept test of intracerebroventricular (ICV) delivery of adult NSCs into the lateral ventricle in an impact-acceleration TBI model with traumatic axonal injury in the corpus callosum. We also test the effect of NSC transplantation in stimulating a regenerative response in endogenous NSCs in the host subventricular zone (SVZ) based on activation of Sonic hedgehog (Shh) signaling. Shh maintains the NSC niche in the adult CNS and Gli1 transcription indicates active Shh signaling. TBI from impact to the skull at bregma was produced in adult Gli1CreERT2 mice crossed to RosaTdTomato reporter mice. To distinguish between endogenous and transplanted cells, NSCs were isolated from the SVZ of adult UBI-GFP mice, which ubiquitously express green fluorescent protein (GFP). GFP-NSCs, 2 µl of 2.5 X 104 cells/µl suspended in F12/DMEM medium, were microinjected unilaterally into the lateral ventricle of the Gli1CreERT2;RosaTdTomato mice 2 weeks after the TBI or sham procedure. Gli1CreERT2;RosaTdTomato host mice were then administered tamoxifen to label endogenous cells responding to Shh signaling after ICV transplantation. Mice were sacrificed for tissue analysis at 4 weeks post-TBI. Analysis of serial tissue sections taken from each mouse showed transplanted GFP-NSCs survived, maintained an immature phenotype, and were localized along the ependymal lining of the ventricle and adhering to the choroid plexus. Endogenous NSCs in the adjacent SVZ were Gli1 fate-labeled with the TdTomato reporter, indicating active Shh signaling. However, Gli1 fate-labeled cells were not changed significantly due to ICV transplantation of GFP-NSCs versus vehicle injection in TBI or sham mice. An inflammatory response to transplanted GFP-NSCs was not observed in sham or TBI mice. Ongoing studies are examining potential effects of the NSC transplant to attenuate neuroinflammation in the corpus callosum after TBI.

Disclosures: G. Sullivan: None. **R.C. Armstrong:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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KSCHIRT 12-20A

Title: A translational approach to the innate immune responses following TBI: exploring the nexus of the post injury proinflammatory cytokine mediated responses with the intracellular p38 MAPK signal transduction pathway

Authors: *L. J. VAN ELDIK[1], S. J. WEBSTER[1], D. S. GOULDING[1], D. M. WATTERSON[2], A. D. BACHSTETTER[1];
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Abstract: Closed head traumatic brain injury (TBI) triggers a broad innate immune and acute inflammation response that involves resident glia and other immune cells. Neurologic outcome is dependent on the essential balance between restoration of tissue homeostasis after the initial injury and resolution of the injury-induced innate immune responses, especially those involving the proinflammatory cytokines that are implicated in both aspects of the neurologic outcome balance. Natural resolution of the injury-induced proinflammatory cytokine response such that neurologic sequelae are attenuated is generally not successful. Therefore, therapeutic interventions during critical dosing time windows are needed in order to reduce the dysregulated inflammation that is causally linked to the neuropathologic sequelae. Previous work has generated a causal link between the p38α mitogen-activated protein kinase (MAPK) mediated intracellular signaling pathway and the injurious proinflammatory cytokine response in neurodegenerative animal models of disease. The recent availability of highly specific *in vivo* molecular probes for p38α MAPK inhibition allow a more refined *in vivo* analysis of this intracellular signaling pathway and its link to dysregulated glia function and neuroinflammation in TBI with its more rapid pathology progression kinetics. We have recently explored these processes in TBI through the combined use of these *in vivo* p38α MAPK dynamic molecular probes and genetics based *in vivo* tools, such as targeted knockdown of p38α MAPK in specific inflammatory cell types. We found that genetic suppression of p38α MAPK in myeloid cells resulted in less TBI induced deficits in a running wheel behavioral task and cognitive deficits as measured by the radial arm water maze. Suppression of p38α MAPK activity through selective

pharmacological action or through reduction of p38α MAPK protein levels generated reduction of injury injury induced cytokine levels in the brain. The congruence of outcomes from genetic and pharmacological approaches provides a unique battery of outcomes consistent with p38aMAPK as a potential therapeutic target in TBI.

Disclosures: L.J. Van Eldik: None. **S.J. Webster:** None. **D.S. Goulding:** None. **D.M. Watterson:** None. **A.D. Bachstetter:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Support: NIH T32 DK059803

Title: Alterations in glutamate regulation after chronic lateral fluid percussion injury

Authors: *J. L. MCGUIRE, E. A. K. DEPASQUALE, A. E. GARDNER, R. E. MCCULLUMSMITH;
Psychiatry, Univ. of Cincinnati, Cincinnati, OH

Abstract: A significant number of patients experience persistent symptoms after brain injury (TBI), which may include deficits in cognition and memory, and changes in personality and behavior. Furthermore it is becoming clear that TBI is a significant risk factor for later emergence of psychological and behavioral disorders and early cognitive decline. In addition to significant contributions to energy homeostasis, glutamate is the major excitatory neurotransmitter in the brain and is utilized by both neurons and glia. Currently, there is a poor understanding of how injury impacts glutamate homeostasis over time. However, TBI increases the risk for developing a number of disorders and behaviors associated with dysregulation of the glutamate system including depression, substance abuse, epilepsy, impulsivity, aggression, and suicidality, suggesting injury-induced changes in glutamate regulation may contribute to chronic TBI symptoms. Glutamate neurotransmitter released into the synapse is taken up primarily by the astrocytic glutamate transporters EAAT1 and EAAT2 (GLT-1 and GLAST, respectively, in rodent). We propose that a persistent deficiency in astrocytic glutamate reuptake accompanies chronic cognitive and behavioral symptoms after TBI. Using the lateral fluid percussion (LFP) model of mild-moderate brain injury in rats, we will measure GLT-1 and GLAST protein levels and glutamate reuptake in membrane vesicle fractions from hippocampus (HPC) and frontal

cortex (FC) 60 days after injury. We will also assess the effects of the glutamate transporter modulator ceftriaxone on post-injury GLT-1 expression, glutamate uptake capacity, and long term memory. The data will provide evidence of the long-term effect of injury on glutamate homeostasis and the role of glutamate dysregulation in chronic cognitive symptoms after TBI. These data will also indicate whether injury-initiated changes in the glutamate system are reversible and suggest new avenues for treatment for injuries already in the chronic stage.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Title: Serine-threonine kinase signaling in traumatic brain injury

Authors: *E. DEPASQUALE[1], J. L. MCGUIRE[1], C. DORSETT[2], C. L. FLOYD[2], R. E. MCCULLUMSMITH[1];
[1]Psychiatry, Univ. of Cincinnati, Cincinnati, OH; [2]Univ. of Alabama Birmingham, Birmingham, AL

Abstract: Traumatic brain injury (TBI) is one of the leading causes of death and disability worldwide. Understanding the biochemical processes that occur during and after TBI would better direct efforts in finding treatments. Previous research suggests that alterations in kinase expression and activity contribute to the injury mechanism of TBI. We used a kinome array to examine the changes in serine-threonine kinase activity in a rat lateral fluid percussion (LFPI) model of TBI in both the prefrontal cortex (PFC) and hippocampus (HPC). We found that 25 substrates in PFC and 15 substrates in HPC showed a 1.15 fold change in phosphorylation compared to sham. Using publically available databases, we mapped protein kinases predicted to target the substrates used in the kinome array. We then utilized random sampling permutation analysis to identify kinases targeting the substrates at a greater frequency than would be expected by chance. Signaling network models linking the kinases identified in the random sampling analysis were constructed using known interactions in the Ingenuity database. In PFC, protein kinase B (AKT), creatine kinase (CK), and p21-activated kinase (PAK) were three of the kinases that were both identified in the random sampling analysis and were major hubs in connecting all of the affected kinases in the signaling network models. In HPC, protein kinase C (PKC), protein

kinase A (PKA), and mechanistic target of rapamycin (mTOR) were similarly identified. We then performed inhibitor studies in the prefrontal cortex using pooled samples for TBI and sham groups with AKT, JNK (c-Jun kinase), and PKC-MEK (mitogen-activated protein kinase kinase) inhibitors. We found that in the presence of AKT inhibitor, the sham group had inhibition of phosphorylation of most substrates in the kinome array compared to the untreated control, where the TBI group showed activation in the presence of the same inhibitor compared to their untreated control. JNK and PKC MEK showed much less divergence in their profiles. These data suggest that there is differential regulation of key kinases that could both account for the biochemical changes observed after TBI, as well as acting as a target for potential treatments of traumatic brain injury in the future.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Support: MOST 103-2314-B-038 -038 -

Title: Deletion of androgen receptor affects the expression of p53 and p62 in mice with traumatic brain injury

Authors: Y.-H. CHEN[1], C.-C. TSENG[2], *L.-Y. YANG[3];
[1]Grad. Inst. of Med. Sci., [2]Dept. of Physiol., [3]Taipei Med. Univ., Taipei, Taiwan

Abstract: Traumatic brain injury (TBI) is estimated to affect 10 million people each year and has become one leading global health issue. The common causes of TBI include traffic injuries, sports accidents, falls, violent fights and war. Severe TBI often leads to death or loss of the ability to work and dependence on long-term healthcare, which places an enormous financial burden not only on the National Healthcare System, but also on the patients themselves and the caring relatives. The pathophysiology of TBI is complicated and remains poorly understood. Therefore, it is critical for us to unveil the detailed mechanisms underlying TBI before we can invent optimal therapeutic treatments for TBI. Evidence has shown that androgens exert a beneficial effect on TBI patients and protect neurons against neurotoxicity. Androgen receptors are widely accepted to mediate the effects of androgens. Nonetheless, the role of androgen

receptor in pathophysiology of TBI remains poorly explored. Published results indicate that TBI increases the expression of p53, but decreases the expression of p62. In this study, we aimed to assess the effect of androgen receptor knockout on the expression of p53 and p62 following TBI using the androgen receptor knockout mice. We employed the cortical impactor to induce TBI and evaluated the expression of p53 and p62 in wild-type mice and androgen receptor knockout mice. Our preliminary results showed that TBI increased the expression of p53 and androgen receptor knockout further enhanced the expression of p53 four hours after injury. In contrast, our preliminary data showed that TBI decreased the expression of p62 and knockout of androgen receptor further reduced the expression of p62 four hours following injury. Our results strongly support the idea that androgen receptor plays an important role in the pathophysiology of TBI.

Disclosures: Y. Chen: None. **C. Tseng:** None. **L. Yang:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Title: Hippocampal neuron loss, white matter damage and behavioral alterations following a fluid percussion injury in mice: a role for CD74?

Authors: *L. A. SHAPIRO[1], A. OBENAUS[2], S. MUKHERJEE[3], R. TOBIN[3], K. NEWELL-ROGERS[3];
[1]Dept Surgery & Neurosurg., Texas A&M Univ. COM, Temple, TX; [2]Loma Linda, Loma Linda, CA; [3]Surgery, Texas A&M Univ. HSC, Temple, TX

Abstract: Traumatic brain injury (TBI) is a growing health concern and increasing evidence suggests that even mild TBIs can lead to profound and long-lasting neurobehavioral and neuropathological deficits. Treatment options are lacking, as is an overall understanding of disease pathogenesis. We have previously demonstrated the pattern of neurodegeneration in the ipsilateral cortex between 1-30 days after a fluid percussion injury (FPI) in mice. We also showed the pattern of ipsi and contralateral astrocyte and microglial activation within these time points. More recently, we demonstrated that antagonism of CLIP, the proteolytic cleavage product of the MHCII-associated, immune response gene-associated molecule, CD74, or deletion of CD74, is neuroprotective after TBI. However, these studies were limited to examination of ipsilateral and contralateral peri-lesion-cortices and lacked examination of neurological deficits. Thus, we expanded on these findings and examined white matter damage at 30 day after FPI, as

well as hippocampal cell loss and behavioral performance on a neurological testing battery. Moreover, we assessed performance on the behavioral battery in mice treated with a CLIP antagonist at 30 min after FPI. The results demonstrate hippocampal cell loss, white matter damage and behavioral deficits following fluid percussion injury. The results also showed that CLIP antagonism improved neurological outcome measures. It is possible that the improvements in contralateral hindlimb gate might simply be due to the reduced lesion size in the peri-lesion motor cortex, it is also possible that antagonizing CLIP and/or CD74 has other beneficial effects on TBI outcomes. Current and future studies will focus on investigating numerous acute and chronic outcomes after FPI and CD74 manipulations.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Title: Proteolytic cleavage of L1CAM is a redundant feature after traumatic brain injury in mice

Authors: *L. S. DANGEL, W. BOBKIEWICZ, A. SEBASTIANI, S. THAL, K. ENDRESS, M. SCHAEFER;
Universitätsmedizin Mainz, Mainz, Germany

Abstract: L1CAM (L1) is a cell adhesion molecule of the immunoglobulin superfamily. It is an important regulator in the developing brain and promotes neural repair and regeneration after injury. It has been shown that L1 is cleaved by various proteases such as ADAM10 and Plasmin *in vitro*. More recently, proteolytic cleavage of L1 has been linked to the pathophysiology of acute and chronic neurodegenerative processes. The overall aim of this study is to characterize the expression and cleavage pattern of L1 after traumatic brain injury (TBI) *in vivo*. In addition, L1 cleavage was investigated in transgenic mouse lines with genetically modified expression or function of ADAM-10 (transgenic overexpressing and dominant-negative) and plasmin activator-inhibitors (PAI-1 and PAI-2 gene-deficient mice). Controlled cortical impact (CCI) was used as an experimental model of TBI. Mice were sacrificed at different posttraumatic time points ranging from one day to seven days after TBI. Gene and protein expression levels were

analysed by qRT-PCR, western blot and immunohistochemistry. Cleavage of L1 was detected as early as one day after trauma, sustained until seven days after trauma and involved consecutive cleavage of membrane-associated L1 including nuclear translocation of a C-terminal fragment. This process was unchanged in mice deficient for the plasmin regulators PAI-1 or PAI-2 as well as in mice with transgenic expression of wild-type or dominant-negative ADAM10. These results demonstrate that TBI-induced L1 cleavage is a redundant feature independent of Plasmin and ADAM10.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Title: Pediatric traumatic brain injury induces selective loss of cortical inhibitory function

Authors: *J. NICHOLS[1,2], J. NEWBERN[1], T. ANDERSON[2];
[1]Arizona State Univ., Tempe, AZ; [2]Basic Med. Sci., Univ. of Arizona - Col. of Med. Phoenix, Phoenix, AZ

Abstract: Pediatric traumatic brain injury (TBI) is a leading cause of death and disability in children. When TBI occurs in children it often results in more severe cognitive and behavioral deficits. Post-injury, the pediatric brain may be particularly sensitive to the effects of TBI while it is undergoing a number of age-dependent physiological and neurobiological changes. The maintenance of proper cortical function is reliant on the activity of inhibitory interneurons that may be more sensitive to neural insult. Therefore, we hypothesize that traumatic brain injury to the cortex will induce selective loss of cortical interneurons and alter inhibitory cortical function. To model TBI we performed controlled cortical impact (CCI) in juvenile (post-natal day 22) mice with fluorescently tagged inhibitory interneurons (VGAT:Cre Ai9). Following a 14 day recovery period no distinct change in overall inhibitory number were observed in either the ipsilateral peri-injury zone or the corresponding contralateral cortex. However, immunohistochemical analysis revealed a greater than 80% loss of parvalbumin (PV) positive interneurons in the peri-injury zone. PV positive interneurons are reported to have a fast-spiking (FS) electrophysiological phenotype and are implicated in a variety of neurological diseases. Patch clamp recordings from FS interneurons revealed a significant decrease to the inhibitory

synaptic network within the peri-injury zone. Specifically, layer V FS interneurons in the peri-injury zone displayed an increase in the inter-event interval, rise, and decay time of spontaneous inhibitory post-synaptic currents (sIPSCs) as compared to recordings from sham animals. In contrast, excitatory activity was enhanced in the peri-injury zone as determined by an increase in the amplitude of excitatory PSCs onto FS interneurons. Taken together, the data suggests that CCI in pediatric animals induces selective loss of PV interneurons that results in a substantial decrease in network synaptic inhibition. Anatomical and synaptic changes to the cortical inhibitory network, particularly parvalbumin neurons, following TBI may lead to abnormal network activity and synchronization. If present in children, TBI induced disruption of the inhibitory network may have a profound impact on their neurodevelopment and the long-term consequences of brain injury.

Disclosures: **J. Nichols:** None. **J. Newbern:** None. **T. Anderson:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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DOD TATRC W81XWH-11-1-0634

Title: Pathological deficits in the chronic stage of traumatic brain injury: characterization of the secondary cell death in adult rats exposed to controlled cortical impact injury

Authors: *H. V. NGUYEN[1], S. A. ACOSTA[2], N. TAJIRI[2], J. HOOVER[2], M. ELIAS[2], S. REYES[2], Y. KANEKO[2], C. V. BORLONGAN[2];
[1]Ctr. of Excellence For Aging and Brain Repair, [2]Ctr. of Excellence for Aging and Brain Repair, Univ. of South Florida, Tampa, FL

Abstract: Long lasting histological consequences have been suggested as accompanying the chronic stage of traumatic brain injury (TBI) and present as a risk factor for the development of dementia and neurodegenerative diseases, such as Alzheimer's disease (AD). The region-specific secondary damage after TBI, specifically at the cellular level of cortical and subcortical regions, is not well characterized. In the present *in vivo* study, the neuronal and axonal damage were

evaluated in proximal and distal areas in late stage TBI model. Recognizing the contribution of AD-like pathology to TBI, we focused on AD pathological markers in brain regions implicated in the disease process, including neuronal cell loss, inflammation, cell proliferation, neurogenesis, and aberrant intra-neuronal and axonal beta-amyloid precursor protein (β-APP) expression, to link TBI and AD pathologies. Adult Sprague-Dawley male rats were subjected to moderate controlled cortical impact (CCI) injury model of TBI, and 6 months later euthanized and brain tissues harvested. Results from H&E staining revealed significant 33% and 10% reduction in the ipsilateral and contralateral side, respectively of hippocampal CA3 interneurons of TBI animals compared to sham animals (p's<0.001). Meanwhile, unbiased stereology showed significant increments in MHCII-activated inflammatory cells in gray matter (8-20 fold) and white matter (6-30 fold) of both the ipsilateral and contralateral hemispheres of TBI animals compared to sham animals (p's<0.001). Cell cycle regulating protein marker revealed significant 1.6 and 1 fold reductions in the SVZ and a 2.3 and 1.5 fold reductions in the dentate gyrus of the ipsilateral and contralateral side, respectively of TBI animals compared to sham animals (p's<0.001). Moreover, immature neuronal marker demonstrated a significant 2 and 1 fold decrements in both the SVZ and in the dentate gyrus ipsilateral and contralateral side, respectively of TBI animals compared to sham animals (p's<0.001). Additionally, we found increased β-APP distribution volume in white matter including corpus callosum, fornix, and internal capsule (4-38 fold), as well as in the gray matter including motor cortex, striatum hilus and dentate gyrus (6-40 fold) in TBI animals compared to sham animals (p's<0.001). These findings indicate AD-like pathological features in brain regions proximal to the core impact of injury in late stage TBI. The observed inflammation, impaired neurogenesis, and β-APP reminiscent of AD may prove to be valuable markers of TBI pathology.

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Poster

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Support: NIH/NIAAA T32 AA01352

NIH/NIAAA-R21 AA020951

Title: Repeated binge alcohol and traumatic brain injury results in decreased neural stem cell response

Authors: *S. T. TON[1], I. VAAGENES[3], S.-Y. TSAI[3], C. PAPADOPOULOS[3], G. KARTJE[3,2]; [1]Loyola Univ. Chicago, Chicago, IL; [2]Neurosci., Loyola Univ. Chicago, Maywood, IL; [3]Hines VA Hosp., Hines, IL

Abstract: We have previously found that a repeated dose of binge alcohol prior to TBI leads to worse recovery on a sensitive test of skilled forelimb function (Vaagenes et al. 2015). One means by which the brain is able to compensate for injury is in the mobilization of neural precursor cells. We therefore sought to determine the effect of binge alcohol at the time of TBI on subventricular zone (SVZ) and perilesional neural precursor cells. Adult, male rats were given three injections of alcohol (2gm/kg/i.p/per day). One hour after the final injection, animals were given a TBI directed to the forelimb sensorimotor cortical area and sacrificed three weeks after TBI. Brains were immunostained for proliferating cell nuclear antigen and doublecortin. In animals receiving binge alcohol there was a significant reduction in PCNA-positive cells in the SVZ. Alcohol also reduced the number of double labeled PCNA-positive/DCX-positive cells on the side of the TBI, but not contralateral to the cortical lesion. Furthermore, binge alcohol significantly reduced the number of PCNA-positive cells as well as double labeled PCNA-positive/DCX-positive cells in the cortical perilesional area. Thus, a relatively short repeated dose of binge alcohol prior to TBI reduced the proliferation of neural precursor cells in the SVZ and perilesional area as well as decreased the differentiation of these cells into neurons. We are currently using alcohol administration by gastric gavage (3gm/kg/per day for three days prior to TBI) to determine the neural precursor cell response in this more clinically relevant model of binge alcohol. Our preliminary results show that at 24 hours post TBI, there is decreased cell proliferation within the SVZ in binge alcohol animals, consistent with the observation made at 3 weeks post TBI in the i.p model. We are currently examining later time points to determine if the decrease in neural stem cells is the underlying mechanism for impaired functional recovery.
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Poster

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State of Indiana

Title: Oxidative stress and inflammation associated with impairments in social familiarity-induced anxiolysis after mild blast-induced traumatic brain injury

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Abstract: Blast-induced traumatic brain injuries (bTBI) are most commonly suffered by military service members. These injuries can occur without noticeable physical signs of injury but can nonetheless be potentially crippling. While extreme cases of bTBI result in early diagnosis, immediate treatment, and monitoring of injury progression, mild bTBI (mbTBI), however, can go unnoticed due to the absence of acute clinical symptoms which result in delayed therapeutic intervention. The development of sensory, communication, cognitive, and behavioral problems is particularly alarming in mbTBI. Despite increased interest in TBI, and bTBI in particular, we are still lacking general pathophysiological information correlating the blast wave pressure component to specific behavioral and cognitive abnormalities. Among various post-bTBI neurological sequela, impairments in social behavior are probably the most difficult to detect and significantly affect social reintegration. We have previously established an animal model of mbTBI, which recapitulates social familiarity-induced anxiolysis (SoFiA), a similar phenomenon. During SoFiA the presence of a conspecific serves as a safety cue that gradually alleviates anxiety-like behavior in response to a threat. However, when rats were subjected to a mbTBI, such anxiolytic ability is impaired. In the presence of an anxiolytic stimulus and a familiar partner, injured animals present an anxiety-like response in the form of low social interaction time. These findings represent the first replication of post-mbTBI social processing impairment in an animal model. We have correlated these behavioral deficits to an early rise in acrolein, an oxidative stress and pro-inflammatory neurotoxin. This suggests that both the primary physical deformation as well as the secondary biochemical cascades work synergistically to damage the brain neurocircuits critical for the integrity of psychosocial capability. The medial prefrontal cortex (mPFC) has been shown to play a pivotal role in SoFiA. Consequently, we have focused our biochemical screening on the mPFC. In the absence of significant neuronal cell death, microglia activation and increased expression of pro-inflammatory cytokines in the mPFC after mbTBI were detected. Interestingly, similar biochemical abnormalities were obtained from animals injected with acrolein at a pathological

relevant concentration. Taken together, these data suggest that acrolein may be an early biomarker for predicting post-mbTBI psychosocial impairment, but could also serve as an effective target for therapeutic intervention.

Disclosures: **S.M. Vega Alvarez:** None. **N. Race:** None. **E. Lungwitz:** None. **T.R. Warner:** None. **G. Acosta:** None. **W. Truitt:** None. **R. Shi:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 43.22/C91

Topic: C.10. Trauma

Support: Medical College of Wisconsin Department of Neurosurgery

VA Medical Research

Title: The acute effects of a blast overpressure on astrocytes in rat organotypic hippocampal slice cultures

Authors: ***A. P. MILLER**[1,2,3], A. S. SHAH[1,3], B. V. APERI[1,3], B. D. STEMPER[1,3], A. GLAVASKI-JOKSIMOVIC[1,2,3];
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Abstract: Blast traumatic brain injury (bTBI) is a significant injury afflicting soldiers and civilians around the world. Symptoms of a bTBI are multifaceted and involve several neuropathological impairments, including learning and memory deficits. The development of novel therapeutic strategies relies on the characterization of the cellular response to a blast injury. Specifically, susceptibility of astrocytes to a blast overpressure and their role in neurodegeneration following a blast injury has yet to be characterized. The aim of this investigation was to characterize the effects of a blast overpressure on astrocytes acutely following a blast injury. Rat organotypic hippocampal slice cultures (OHCs) were used to generate an *in vitro* bTBI model. The hippocampi obtained from neonatal Sprague Dawley (SD) rats (p7-p10) were cut into 400 μm thick sections and grown using the membrane interface method. At 8 days in culture, a blast injury was produced using an open-ended gas-driven shock tube. Sections were exposed to a single blast overpressure of either 147 ± 18 kPa (low) or 278 ± 22 kPa (high). Sham injured sections were treated identical to blast injured sections, except

the shock tube was not detonated. Sections were fixed 2 h following injury and the astrocyte morphology was characterized using immunostaining against glial fibrillary acidic protein (GFAP). Additionally, dead astrocytes were visualized by co-labeling of GFAP and the cell death marker propidium iodide (PI). At 2 h following injury, in both blast groups we detected shearing of astrocytes as well as clasmatodendrosis, which is characterized by cytoplasmic swelling, beading and dissolution of distal astrocytic processes. These morphological changes in astrocytes were infrequent in the sham-injured OHCs. Our preliminary quantification of GFAP and PI co-labeled cells in the CA1 hippocampal region revealed a significant increase in the number of dead astrocytes in the low- ($P<.01$) and high-blast ($P<.001$) group compared to the sham-injured group. Collectively, our data imply astrocytes are affected acutely following overpressure exposure both in the low- and high-blast group. Future studies will elucidate whether there is a correlation between the effects of a blast overpressure on astrocytes and delayed blast-evoked neuronal death.

Disclosures: A.P. Miller: None. **A.S. Shah:** None. **B.V. Aperi:** None. **B.D. Stemper:** None. **A. Glavaski-Joksimovic:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Support: State of Indiana

Title: Susceptibility to Parkinson's disease following a mild-blast traumatic brain injury

Authors: *G. G. ACOSTA[1], N. RACE[2,5], G. KUZIEL[2], L. ZHENG[2], A. AMBAW[1], E. WALLS[3], C. ROCHET[4], R. SHI[2,1];
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Abstract: The prevalence of blast-induced traumatic brain injury (bTBI) is steadily increasing due to escalated terror activities, and constitutes the signature injury associated with the current military conflicts. Specifically, a mild-bTBI is the most common injury encountered by our military personnel. This type of injury presents a problem because the individual is initially asymptomatic and functional. However, growing evidence suggests that this type of injury may

produce long-term neurological consequences that affect the resilience and the performance of soldiers both on and off the battlefield. One such example is an increased susceptibility to Parkinson's disease (PD) by as many as three folds post-blast when compared to the general population. A critical strategy aiming at curtailing this alarming trend is to further our knowledge of pathogenic mechanisms responsible for the escalation of post trauma neurodegenerative diseases. The specific aim of this investigation was to identify the molecular mechanisms underlying the susceptibility to PD in post-blast rats. To this end, we have identified acrolein, a highly reactive aldehyde that persists days to weeks following CNS injury and perpetuates oxidative insult, as a point of convergence between bTBI and PD. Specifically, we have found that the elevation of acrolein post-blast is capable of triggering pathological changes in the vicinity of the basal ganglion, a known location of brain damage in PD. In particular, we have found signs of neuroinflammation and protein aggregation in blast animals that resembles the pathology in PD, although to a lesser extent. In addition, although a mild blast injury alone cannot elicit typical motor deficits seen in a PD model, additional application of a subthreshold PD-inducing toxin could lead to such deficits. Taken together, we hypothesize that bTBI triggers neurochemical events, such as neuroinflammation and oxidative stress, galvanized by acrolein, could increase the susceptibility of the blast-injured rats to PD when other PD-triggering factors are present. As such, we hypothesize that acrolein is a key pathological factor linking bTBI and the development of PD in our rat model. The results from this project are expected to advance our understanding of the long-term consequences of blast-related injuries leading to the development of PD. These efforts could eventually lead to the establishment of biomarkers for an earlier diagnosis as well as strategies for prevention and treatment to curtail the elevating incidence of post-bTBI PD, and significantly improve the quality of life for our men and women who suffer a great deal to ensure our freedom.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Support: Career development grant [G1708X] to MKJ

CNRM grant [G1703O] to ZG

Title: In-vivo 2-photon imaging of neuronal activity and neuro-vascular unit disruption in somatosensory cortex in mouse model of traumatic brain injury

Authors: *M. K. JAISWAL[1,2], F. W. LISCHKA[1,2], X. XU[2], Z. GALDZICKI[1,2];
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Abstract: The adult brain must undergo plasticity driven events to respond to injury or insult. In response to TBI the functional somatosensory topography of the neocortex is altered such that cortical areas or 'maps' of severed inputs shrink, while maps of the spared inputs expand. However, it is not known how sensory-driven activity in individual L2/3 neurons changes over time after brain injury, and how these changes impact local excitatory/inhibitory (E/I) neuronal populations in response to injury. Moreover, the relationships between alterations in plasticity and vasculature disruption after injury have not been fully understood. We attempted to address these questions using our mild TBI open head model with injury inflicted by CCI device over somatosensory region. Activity of E/I neurons was monitored by *in vivo* two-photon calcium imaging in GAD67-GFP knock-in mice. In our approach we assessed the loss/gain of spontaneously evoked neuronal activity in the core of the microinfarct at 2 weeks and 6 months after injury. We examined changes in vasculature and integrity of the blood-brain barrier with combination of dyes: Rhodamine B dextran, Cascade blue or Texas red concavalin. We also performed simultaneous recordings of Ca2+ transients in E/I neurons, which revealed a decrease of spontaneous activity within L2/3 neuronal population in proximity of injury epicenter. In order to determine functional impact at 6 months post injury we measured evoked L2/3 neuronal activity by imaging the hindlimb region of the primary somatosensory cortex, where peripheral electrical stimulation reliably activated a large number of neurons. The number of excitatory neurons responsive to hindlimb stimulation appears to decrease in the injured area. These changes might be caused directly by injury impact or indirectly though time-dependent remodeling processes over 6 month period after injury. Overall, our preliminary results suggest reorganization of cortical population activity with vascular defects likely exacerbating neuronal deficits.

Disclosures: **M.K. Jaiswal:** None. **F.W. Lischka:** None. **X. Xu:** None. **Z. Galdzicki:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Support: CDMRP grants W81XWH-08-2-0018 and W81XWH-11-2-0127 to JBL

Title: Tryptophan metabolism after blast-induced traumatic brain injury

Authors: *P. ARUN, D. M. WILDER, W. B. RITTASE, M. A. MCCUISTION, S. A. OGUNTAYO, Y. WANG, I. D. GIST, J. B. LONG;
Ctr. for Military Psychiatry and Neurosci., Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Blast-induced traumatic brain injury (bTBI) is one of the major disabilities in service members returning from recent military operations. The neurobiological underpinnings of bTBI, which are associated with acute and chronic neuropathological and neurobehavioral deficits, are uncertain. The role of the essential amino acid tryptophan in the pathogenesis of bTBI has not been explored. We have reported earlier that blast exposure causes significant decrease in the levels of the neurotransmitter and tryptophan metabolite serotonin, which has been implicated in affective disorders such as depression and anxiety. In this study, rats and mice were subjected to single or repeated blast overpressure waves using a compressed air-driven shock tube. At different intervals after blast exposures, the animals were euthanized and brain, spleen and blood plasma were collected. The expression or levels of indoleamine 2,3-dioxygenase (IDO1) in different brain regions, spleen and plasma were determined by Western Blotting using rabbit monoclonal antibodies. Brain serotonin and plasma tryptophan levels were determined by ELISA. Our preliminary results revealed up-regulation of IDO1 in different regions of the brain after blast exposure which increased with repeated blasts. Blast exposure acutely increased expression of IDO1 in the spleen and the expression was further elevated with repeated blast exposures. Analysis of plasma samples indicated increased levels of IDO1 and decreased levels of tryptophan. The up-regulation of IDO1 in the brain after blast exposure, which may be an endogenous immunosuppressive protective mechanism mediated through the kynurenine pathway, could account for decreased levels of brain serotonin. The increased levels of IDO1 in the spleen and plasma may be responsible for the depletion of plasma tryptophan, which may also responsible for the decreased synthesis of serotonin in the brain. These results reveal that systemic and central tryptophan metabolism are disrupted following blast exposure which might play a significant role in the pathogenesis of neurobehavioral deficits associated with bTBI.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 43.26/C95

Topic: C.10. Trauma

Title: Repetitive mild traumatic brain injury induces ventriculomegaly and cortical thinning in juvenile rats

Authors: C. GODDEYNE[1,2], J. NICHOLS[1,2], C. WU[1], K. MAGNUSON[1], Z. KILLEEN[1], *T. R. ANDERSON[3];
[1]Univ. of Arizona - Col. of Med. Phoenix, Phoenix, AZ; [2]Arizona State Univ., Tempe, AZ; [3]Univ. of Arizona-Com PHX, Phoenix, AZ

Abstract: Traumatic brain injury (TBI) most frequently occurs in pediatric patients and remains a leading cause of childhood death and disability. Mild TBI (mTBI) accounts for nearly 75% of all TBI cases, yet the underlying neuropathophysiology remains poorly understood. While even a single mTBI injury can lead to persistent deficits, repeat injuries increase the severity and duration of both acute symptoms and long term deficits. To model pediatric repetitive mTBI (rmTBI) we subjected unrestrained juvenile animals (post-natal day 20) to repeat weight-drop impacts. Animals were anesthetized and subjected to sham or rmTBI once per day for 5 days. Biomechanics of injury were measured using accelerometers, force meters and high-speed video. Magnetic resonance imaging (MRI) performed 14 days after injury revealed marked cortical atrophy and ventriculomegaly in rmTBI animals. Specifically, beneath the impact zone the thickness of the cortex was reduced by 46% and the area of the ventricles increased 970%. Immunostaining with the neuron-specific marker NeuN revealed an overall loss of neurons within the motor cortex but no change in neuronal density. Examination of intrinsic and synaptic properties of layer II/III pyramidal neurons revealed no significant difference between sham and rmTBI animals at rest or under convulsant challenge with the potassium channel blocker, 4-Aminophyridine. Overall, our findings indicate that the neuropathological changes reported after pediatric rmTBI can be effectively modeled by repeat weight-drop in juvenile animals. Developing a better understanding of how rmTBI alters the pediatric brain may help improve patient care and direct "return to game" decision making in adolescents.

Disclosures: **C. Goddeyne:** None. **J. Nichols:** None. **C. Wu:** None. **K. Magnuson:** None. **Z. Killeen:** None. **T.R. Anderson:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Topic: C.10. Trauma

Support: NIH Grant R21NS090092

Title: Septal and periventricular changes in a mouse model of repetitive concussive traumatic brain injury

Authors: *R. ACABCHUK[1], R. WOLFERZ, Jr.[1], L. TALBOT[1], M. STERM[1], M. SOLOWAY[1], M. ANGOA-PEREZ[2], D. BRIGGS[2], D. KUHN[2], J. CONOVER[1];
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Abstract: Contact sports, such as football and boxing, significantly increase the risk of exposure to repetitive concussive traumatic brain injury (rcTBI), a public health concern reaching epidemic proportions, and repeated concussive impacts have been linked to long-term neurodegenerative pathology including chronic traumatic encephalopathy (CTE). Recently, a wide array of rodent models have been used to examine the cellular, molecular and behavioral sequelea resulting from rcTBI, with the majority of these studies focusing primarily on the hippocampus, amygdala and cortex. However, the septal and periventricular regions can also incur long-term damage from repeated exposure to concussions as revealed in post-mortem tissue analysis of patients diagnosed with CTE. Cavum septum pellucidum and expansion of the lateral ventricles are hallmark features of CTE. To investigate pathological changes in the lateral septum and periventricular regions following rcTBI, we utilize an unrestrained closed-skull impact mouse model that allows for 180^{o} free rotation following impact to mimic concussive forces found in humans. Regional and temporal alterations in astrogliosis, microglial activation, water channel protein expression, axonal damage, and phospho-tau levels are investigated to provide a detailed analysis of histological changes incurred following rcTBI. For our studies, two strains of mice (CD1 and C57/Bl6) were examined using multiple inter-injury intervals ranging from 5 hits in 3 days to 5 hits in 2 weeks. Our results demonstrate that immediate and persistent cellular alterations occur in the septum and periventricular region in both CD1 and C57/Bl6 mice. Together, these studies highlight the septum and periventricular regions as areas of interest in rcTBI research.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

Location: Hall A

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Topic: C.10. Trauma

Support: VA Grant I01 RX000952

Title: Blast injury increases amyloid-beta deposition in cortical areas but not in subcortical visual pathways in young adult APP/PS1 mice

Authors: E. E. ABRAHAMSON[1,2], Z. MI[1,2], L. SHAO[1], D. S. RUDD[3], M. M. HARPER[3], *M. D. IKONOMOVIC[1,2];
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Abstract: Blast overpressure results in impaired retinal and higher-order visual functions. Because brain trauma is a risk factor for Alzheimer's disease (AD), we hypothesized that blast injury-induced visual dysfunction is associated with accumulation of β-amyloid (Aβ) peptide in the retina and visual pathways. We examined Aβ-immunoreactive aggregates in the retina, optic tract, lateral geniculate nucleus, primary visual cortex, and posterior parietal association/somatosensory/auditory cortices of adult APPswe/PS1ΔE9 (APP/PS1) transgenic mice (aged 83-106 days at injury; 150-175 days at sacrifice, when Aβ plaques develop in this model), after blast injury (unrestrained, head-only single blast wave to left side; ~150 kPa, 10-15 ms) compared to sham animals. In retina, small, diffuse extracellular plaques were infrequently observed in a subset of sham and blast exposed APP/PS1 mice, with no clear differences between groups. However, in blast, but not sham-surgery-exposed mice, we detected intense Aβ (4G8) immunoreactivity in blood vessels in the ganglion cell layer and inner and outer plexiform layers. No Aβ immunoreactivity was observed in the optic tract or lateral geniculate nucleus in either group. Cortical Aβ plaque load in primary visual cortex was comparable to posterior parietal association/somatosensory/auditory cortices in sham mice (<0.2% area coverage). In blast-exposed mice, plaque load values in all cortical regions were higher than in sham mice, with a two-fold increase in the posterior parietal association-somatosensory-auditory cortices compared to primary visual cortex. These results suggest that blast injury accelerates Aβ plaque development differentially across cortical regions in APP/PS1 mice. The less prominent increase in Aβ plaque pathology in the primary visual cortex might be due to de-afferentation or reduced synaptic activity/Aβ release in injured central visual pathways. Retina, optic tract, and lateral geniculate nucleus appear to be spared of intra- and extra- cellular accumulation of APP/Aβ in blast-injured APP/PS1 mice at the examined age and injury-to-death interval, despite substantial anterior afferent functional deficits compared to sham animals.

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Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.10. Trauma

Title: A novel, quantitative, multi-analyte immunoassay to detect neuroinflammation following traumatic brain injury

Authors: M. ANDERSON, *G. HICKEY, I. O'BRIEN, P. YOUNGE, L. LEONG;
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Abstract: There is an increasing public awareness of Traumatic Brain Injury (TBI) that can be attributed to its appearance in widely diverse age groups that range from children, to military personnel, to retirement age individuals. This has created a need for the early detection of circulating biomarkers that reflect a traumatized state. Neuronal injury typically stimulates the release of pro-inflammatory mediators that induce a neuro-inflammatory response. Presumably this response is beneficial in the end, as it lays the groundwork for subsequent tissue repair and restoration. By contrast, a prolonged, unregulated release of pro-inflammatory cytokines and chemokines has the potential to damage uninvolved neurons and compromise brain function. The ability to distinguish between short-term and prolonged, or long term, inflammation is thus desirable, particularly when using a marginally-invasive method that utilizes serum or plasma. The study presented here evaluated several neuro-inflammatory markers including; CHI3L1/YKL40, ICAM-1, IL-1β, IL-6, IL-8, IL-10, IP-10 and TNF-α. The Simple Plex™ assay employed is a novel, quantitative, multi-analyte immunoassay platform that delivers high precision and accuracy all from ≤ 25 uL of sample. This platform measures up to four analytes simultaneously from a single, small sample with very high sensitivity. The microfluidic-based system allows parallel single analyte detection, and reduces non-specific antibody interactions that are often observed in other traditional multiplex platforms. The assay is a closed system, removing potential user variability. This is accomplished by automating the entire assay process within a single cartridge that provides results in just over one hour. Our studies suggest this platform may represent a highly efficient method for the detection of pro- and anti-inflammatory cytokines following TBI, and may also have relevance to chronic neurodegenerative diseases

such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis.

Disclosures: **M. Anderson:** None. **G. Hickey:** None. **I. O'Brien:** None. **P. Younge:** None. **L. Leong:** None.

Poster

043. Traumatic Brain Injury: Cellular and Molecular Mechanisms

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Support: NJCBIR CBIR11PJT003 to K.P and V.S.

NJCBIR CBIR14RG024 to V.S

F.M. Kirby Foundations to V.S

CURE to V.S

Title: Neurological consequences of mechanistically distinct toll-like receptor 4 signaling in the normal and injured brain

Authors: *A. A. KORGAONKAR[1], K. C. H. PANG[1,2], V. SANTHAKUMAR[1];
[1]Dept. of Pharmacology, Physiol. and Neurosciences, Rutgers New Jersey Med. Sch., Newark, NJ; [2]Dept. of Veteran Affairs Med. Center–New Jersey Hlth. Care Syst., Neurobehavioral Res. Lab., East Orange, NJ

Abstract: Traumatic brain injury (TBI) is a leading cause of acquired epilepsy and memory deficits. TBI causes both early activation of immune responses and increase in network excitability in the hippocampal dentate gyrus. Recently, we showed that enhanced neuronal expression of the innate immune receptor, Toll-like Receptor 4 (TLR4) contributes to increase in dentate excitability after TBI (Li et al., 2015). Unexpectedly, TLR4 agonists reduced excitability in uninjured rats. Here, we examine the cellular mechanisms underlying differential TLR4 effects on the excitability of normal and injured dentate and investigate the effect of TLR4 antagonism on seizure thresholds and working memory of uninjured and injured rats. Wistar rats (25-27 day old) were subject to moderate (2 atm) lateral fluid percussion injury (FPI) or sham procedures. Field recordings in hippocampal slices, video-EEG monitoring and memory studies were conducted at various time points after FPI. Afferent-evoked dentate population spike

amplitude *in vitro* was decreased following incubation in TLR4 antagonist (CLI095) 1 week after FPI, yet, was paradoxically increased in controls. Treatment with glial metabolic inhibitors (fluoroacetate and minocycline) failed to block CLI095-mediated suppression of excitability after FPI but selectively eliminated enhancement of dentate excitability by CLI095 in controls. A single dose of a TLR4 antagonist (LPS-RS or CLI095), administered 24 hours after FPI, prolonged the latency for seizures following kainic acid (5mg/kg, i.p.) injection one (vehicle: 16.2±2.09, LPS-RS: 180±0 min. in 8 rats each) and three months after FPI. However, similar to effects *in vitro*, TLR4 antagonists reduced the latency and promoted kainic acid-induced seizures in uninjured rats (vehicle 55.2±5.48, LPS-RS 15.6±1.38 min., in 8 rats each). Working memory performance (path efficiency) in a delayed match to sample task in the Morris Water Maze showed deficits up to one month after FPI. LPS-RS improved memory performance in rats 1 week and 1 month after FPI without adversely effects in controls. Our data show that glial signaling is necessary for TLR4 modulation of neuronal excitability in controls but not after FPI suggesting that the divergent neurophysiological effects of TLR4 in the normal and injured brain are mechanistically separable. While early post-injury TLR4 antagonism alleviates long-term behavioral deficits, the adverse outcome of the treatment in controls suggests that selectively targeting the processes underlying pathological TLR4 signaling may be efficacious in reducing epilepsy and memory impairments after TBI.

Disclosures: A.A. Korgaonkar: None. **K.C.H. Pang:** None. **V. Santhakumar:** None.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

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Topic: C.10. Trauma

Support: NIH R01 HD061504

NIH U54 EB020403

NIH R01 EB008432

NIH R01 AG040060

NIH R01 NS080655

UCLA BIRC

NIH NS027544

Title: Callosal function in pediatric traumatic brain injury linked to disrupted white matter integrity

Authors: *E. L. DENNIS[1], M. ELLIS[2,4], S. MARION[4], Y. JIN[1], C. KERNAN[2], T. BABIKIAN[2], R. MINK[5], C. BABBITT[6], J. JOHNSON[7], C. GIZA[8], R. ASARNOW[2,3], P. THOMPSON[1];
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Abstract: The corpus callosum (CC) is the most widely reported area of disruption in traumatic brain injury (TBI). Here we present data linking disrupted CC integrity to slowed callosal function in pediatric moderate/severe TBI in the post-acute phase (1-6 months post injury). We examined 63 participants: 32 TBI (mean age = 14.2, 9 female), and 31 controls (mean age = 14.9, 14 female). We used visual ERPs (event related potentials) to measure IHTT (interhemispheric transfer time, msec). EEG was recorded during a visual pattern matching task. To assess white matter integrity, we used a method developed in our lab, autoMATE (automated multi-atlas tract extraction), to generate along-tract measures of fiber integrity from HARDI data (high angular resolution diffusion imaging). The distribution of IHTTs was bimodal within the TBI group: some had IHTTs within 1.5 SD from the control mean, but others differed significantly (group cut-off = 18 msec min). We ran an element-wise linear regression testing for differences in FA and MD between the IHTT-slow TBI group and controls, and the IHTT-normal TBI group and controls. IHTT-slow and control groups differed significantly: the IHTT-slow group had lower FA and higher MD across large areas of the CC and beyond. There were minimal differences in FA between the IHTT-normal and control groups, and none in MD. This divergence was also displayed in neurocognitive function - the IHTT-slow TBI group performed more poorly than IHTT-normal TBI, who performed slightly poorer than healthy controls. This study reveals that there is a subset of pediatric msTBI patients during the post-acute phase of injury who have markedly impaired CC functioning and structural integrity that is associated with poor neurocognitive functioning.

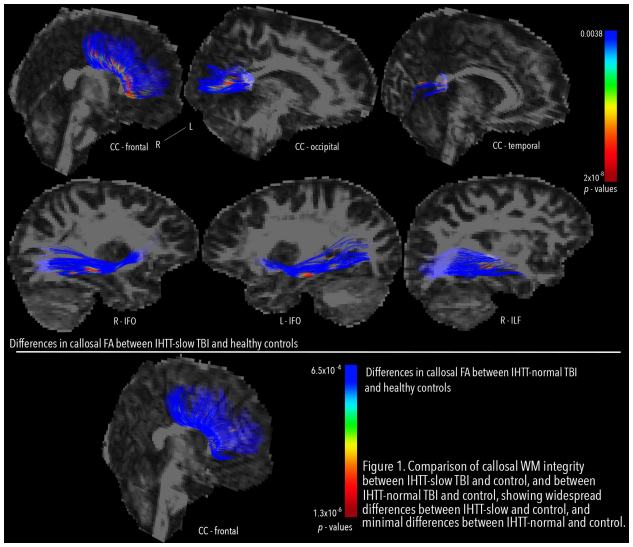

Figure 1. Comparison of callosal WM integrity between IHTT-slow TBI and control, and between IHTT-normal TBI and control, showing widespread differences between IHTT-slow and control, and minimal differences between IHTT-normal and control.

Disclosures: **E.L. Dennis:** None. **M. Ellis:** None. **S. Marion:** None. **Y. Jin:** None. **C. Kernan:** None. **T. Babikian:** None. **R. Mink:** None. **C. Babbitt:** None. **J. Johnson:** None. **C. Giza:** None. **R. Asarnow:** None. **P. Thompson:** None.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 44.02/D5

Topic: C.10. Trauma

Support: University of Western Ontario, Schulich School of Medicine and Dentistry Dean's Research Initiative Grant.

Title: Identification of signature biomarkers in adult female athletes following mild traumatic brain injury

Authors: *K. A. BLACKNEY[1,3], L. FISCHER[5], T. DOHERTY[4], R. MENON[2], R. BARTHA[2], D. FRASER[6], A. BROWN[1], G. DEKABAN[1,7];
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Abstract: Sports-related mild traumatic brain injuries, also known as concussions, are generating increasing concern due to potential long-term neurological consequences. However, there is currently no universally recognized diagnostic approach for concussion, and many of the current methods used for diagnosis are sub-optimal. This leaves patients with insufficient treatment and at enhanced risk of more severe damage from subsequent head trauma. We hypothesize that a signature temporal response of biomarkers of inflammation in systemic circulation will provide an objective concussion diagnostic. We predict that this panel of biomarkers could also be used to track patient recovery. The Western University Women's Rugby Team was the selected cohort as they are an understudied, concussion prone group, and they have been followed over 3 playing seasons. Participants underwent a set schedule of pre-season and post-season baseline evaluations including currently used concussion assessment tools (SCAT III and ImPACT), blood analysis, and a physical examination. Concussed players underwent serial evaluations at 24-72 hours, 1 week, 1 month, and 3 months post-concussion. Importantly, the inclusion of baseline assessments allowed each player to act as their own control in the study. A portion of the blood samples collected was used to determine the hematologic profiles. The remaining blood was separated into leukocytes, plasma, and serum. Plasma samples were analyzed for cytokine levels, as well as the neurotrauma marker glial fibrillary acidic protein (GFAP), and inflammatory marker C-reactive protein (CRP) using ELISA or Luminex methodology. Preliminary analysis showed that currently used concussion assessment tools failed to make an objective diagnosis. Pairwise analysis of players' hematology profiles from baseline to post-concussion show acute (1-5 days), and sub-acute (8-15 days) significant increases in systemic total leukocyte counts. These leukocyte increases positively correlated with symptom scores reported by concussed individuals. No signature changes were detected for the cytokine, GFAP, or CRP concentrations analyzed in the plasma of the concussion participants compared to their uninjured baseline level. A detailed flow cytometry analysis of leukocyte subsets and quantification of systemic inflammatory markers and their temporal response to concussion will be presented. The results of this multifactorial blood analysis would ideally reveal a constellation of biomarkers that could objectively indicate a concussion and determine an individual's progression towards recovery.

Disclosures: K.A. Blackney: None. **L. Fischer:** None. **T. Doherty:** None. **R. Menon:** None. **R. Bartha:** None. **D. Fraser:** None. **A. Brown:** None. **G. Dekaban:** None.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 44.03/D6

Topic: C.10. Trauma

Title: Traumatic Brain Injury and its implication for Anaesthesia

Authors: *Y. O. OKUNOREN-OYEKENU, A. OYEKENU, P. EKWERE, M. O. DAWODU, O. OKUNOREN, A. OLAWUYI, A. AGBOOLA;
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Abstract: Traumatic brain injury (TBI) has become of public health concern as it has been observed to be a leading cause of death and disability globally. Every year reported figures of TBI are in the range of 1.7million for America and greater than 1million in European Union for hospital admission. World Health Organisation (WHO) records of daily figures of about 300 TBI cases in Africa leading to death has led to the establishment of Ondo State Trauma and Surgical Center, a new center in a remote part of Nigeria where motorcycles are the major means of transportation. Since inception around 2013 September, It has recorded 13 cases of TBI within a year which accounts for about 40% of the total number of brain and spinal cord injury cases requiring general anaesthesia. TBI cases are mainly classified as primary or secondary brain injury. The primary brain injury is due to direct impact to the brain leading to fracture, axonal injury, and disruption to the blood brain barrier while the secondary brain injury sequential to the primary brain injury occur via pathophysiologic processes like brain ischemia, inflammatory and cytotoxic processes with hypoxaemia, hypotension and hypoglycaemia leading to an increased risk of secondary brain injury. Anaesthetic management controls intracranial pressure, hypotension and hypoxaemia to avoid secondary brain injury, studies have however shown that anaesthesia negatively contributes to secondary injury in TBI cases. Anaesthesia is employed so that patients can be unconscious during surgery, as TBI patients are already unconscious before surgery arguments arise to the necessity of anaesthesia in TBI cases. The free radical gas Nitric Oxide (NO) is a molecule that has been implicated in brain injury cases for pathophysiolologic and therapeutic actions. Studies by Straub *et al* 2013, have shown that NO signalling affects synaptic remodelling and neuronal regeneration after axonal injury in *Lymnaea stagnalis* (The great pond snail) neurons while Terpolilli *et al* 2013 have proved that Inhaled NO reduces secondary brain damage after traumatic brain injury in mice. This study will therefore investigate if the secondary negative results of TBI cases after neurosurgery are caused by the original trauma or side effects of anaesthesia. We also intend to determine the role and level of expression of NO after Traumatic brain injury and whether therapeutic intervention with inhaled NO can be employed in TBI cases. **Grant Funding; Self-Funded Researchers Full Article will be published in the British Journal of Anaesthesia**

Disclosures: Y.O. Okunoren-Oyekenu: None. **A. Oyekenu:** None. **P. Ekwere:** None. **M.O. Dawodu:** None. **O. Okunoren:** None. **A. Olawuyi:** None. **A. Agboola:** A. Employment/Salary (full or part-time):; ondo state trauma and surgical center, Nigeria.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 44.04/D7

Topic: C.10. Trauma

Support: VA Research Services

Title: Prevalence of memory loss following traumatic brain injury from blast versus non-blast exposure in a veteran population

Authors: *J. RAMOS[1], K. L. PANIZZON[2], A. PAPAZYAN[3], W. STEFANOS[3], J. WATSON[3], E. A. LICHT[4], R. A. WALLIS[3,5];
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Abstract: OBJECTIVE: To evaluate the prevalence of memory loss in Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) veterans with blast versus non-blast traumatic brain injury (TBI). BACKGROUND: TBI is a signature injury for OEF/OIF veterans. Memory loss is a frequent residual deficit after TBI. METHODS: We conducted a retrospective chart review of TBI patients in the Poly-Trauma Clinic of the VA Greater Los Angeles Healthcare System. We collected data about blast versus non-blast exposure in OEF/OIF veterans with memory loss and confirmed TBI. RESULTS: Of 563 charts reviewed, 377 were OEF/OIF veterans with a confirmed TBI diagnosis. The racial/ethnic distribution of subjects was 44 % Caucasian, 12% African-American, 25% Hispanic, 12% Asian and 7% other. The mean age of subjects was found to be 33 ± 1 and 32 ± 1 yr. for blast and non-blast TBI. Of 377 subjects with TBI, a mean 63 ± 3% (n = 237) were noted to have memory loss. In subjects with post-TBI memory loss, 51 ± 3% (n = 121) had blast-TBI, and 49 ± 5% (n = 116) had non-blast TBI. CONCLUSION: These data suggest that post-TBI memory loss is a frequent occurrence in OEF/OIF veterans, with both blast and non-blast-TBI exposure.

Disclosures: J. Ramos: None. **K.L. Panizzon:** None. **A. Papazyan:** None. **W. Stefanos:** None. **J. Watson:** None. **E.A. Licht:** None. **R.A. Wallis:** None.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 44.05/D8

Topic: C.10. Trauma

Support: DoD Grant

Title: Characterization of the epigenomic status of the us oef/oif war veterans: a pilot clinical study

Authors: N. CHAKRABORTY[1], R. YANG[2], S. MUHIE[2], A. GAUTAM[1], D. DONOHUE[1], *J. L. MEYERHOFF[3,1], D. AMARA[4], R. YEHUDA[5], C. MARMAR[4], R. HAMMAMIEH[1], M. JETT[1];
[1]USACEHR, Fredrick, MD; [2]Advanced Biomed. Cancer Research, NCI, Frederick,, MD; [3]Psychiatry & Biochem., Georgetown Univ., Silver Spring, MD; [4]Steven and Alexandra Cohen Veterans Ctr. for the Study of Posttraumatic Stress and Traumatic Brain, NYU Sch. of Med., New York, NY; [5]Dept. of Psychiatry,, Mount Sinai Sch. of Med., New York, NY

Abstract: Management of post-traumatic stress disorder (PTSD) is complicated by the overlapping symptoms of its comorbidities and the diagnostic reliance on self-report and time consuming psychological evaluation. A more comprehensive understanding of molecular pathophysiology of PTSD could facilitate an unbiased biomarker-driven next-generation intervention strategy. Herein, we cast light on the epigenomic consequences of combat elicited PTSD. In this study, hypermethylated genes were investigated as to the implications for behavior, immune response, nervous system development, and relevant PTSD co-morbidities such as cardiac disease and diabetes. 52 PTSD-positive male veterans of US Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) were matched to 52 controls by age and ethnicity. PTSD diagnosis was determined by a clinician-administered PTSD scale (CAPS), score >40, while the control group demonstrated a CAPS <10. Methylation status of DNA extracted from whole blood was assayed using high density arrays (Agilent, Inc.). 5,000 probes were statistically differentially methylated (FDR < 0.1), representing approximately 3,600 unique genes. Chromosome 4 and 18 imprints a significantly large portion of the methylated probes, including those which control emotional and cognitive processes, and glucocorticoid deficiency. Interestingly, a significant number of genes facilitating telomere maintenance and insulin reception were hypermethylated at both promoter and gene body sites; therefore the DNA methylation status in these genes could be a significant factor. Nearly 85% of the differentially methylated probes were hypermethylated in PTSD patients. The majority of these probes encode the candidate proteins responsible for transcription regulation and enzymatic actions. Genes involved in memory consolidation, emotion/aggressive behavior, and perturbed circadian rhythm were preferentially hypermethylated. PTSD epigenetically perturbed both the cellular and humoral immune systems. In addition, the morphologies of two brain regions known to control PTSD symptoms, namely cerebral cortex and hippocampus were perturbed. Genes associated

with several PTSD comorbidities, such as cardiomyopathy and poor insulin regulation, were also hypermethylated. Integration of the epigenomic observations with other omics outcomes is currently underway, as well as validation of these findings in an independent cohort.

Disclosures: N. Chakraborty: None. **R. Yang:** None. **S. Muhie:** None. **A. Gautam:** None. **D. Donohue:** None. **J.L. Meyerhoff:** None. **D. Amara:** None. **R. Yehuda:** None. **C. Marmar:** None. **R. Hammamieh:** None. **M. Jett:** None.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 44.06/D9

Topic: C.10. Trauma

Support: Edward Hines Jr VA, CINCCH LIP 42-129

VA RR&D CDA-II RX000949-01A2

Title: Gray matter density and volume neuroimaging characterization of U.S. Military Veterans and retired National Football League players vulnerable to mild traumatic brain injury

Authors: *A. A. HERROLD[1,2], B. C. HARTON[5], X. WANG[3], T. PARRISH[3], Y. CHEN[3], J. L. REILLY[2], H. C. BREITER[2,6], T. L. B. PAPE[1,4];
[1]Edward Hines Jr. VA Hosp., Hines, IL; [2]Psychiatry & Behavioral Sci., [3]Radiology, [4]Physical Med. & Rehabil., Northwestern University, Feinberg Sch. of Med., Chicago, IL; [5]Chicago Assn. for Res. and Educ. in Sci., Hines, IL; [6]Radiology, Massachusetts Gen. Hosp., Boston, MA

Abstract: United States Military Veterans and National Football League (NFL) players represent two populations at elevated risk for mild traumatic brain injuries (mTBI) and mental health disorders (MHD). Impaired cognitive domains associated with mTBI and MHD include working memory, attention and executive function. The dorsolateral prefrontal cortex (DLPFC) supports these cognitive functions and existing evidence indicates that functional activation of the DLPFC is compromised with mTBI. However, less is known regarding the structural morphometry of the brain among those with mTBI and/or MHD. The purpose of this study was to characterize structural morphometry among a sample of Veterans and retired NFL players relative to healthy participants. Our samples of 9 Veterans, 9 retired NFL players and 9 healthy participants all completed a 3D T1-weighted volume MRI scan (MPRAGE). Veterans and retired NFL players completed neuropsychological testing procedures and mental health symptom

inventories prior to the scan. Veterans endorsed significantly higher Beck Anxiety Inventory scores ($p<0.05$) and Wisconsin Card Sorting Test total and perseverative errors T-scores ($p<0.05$) relative to retired NFL players. Gray matter density analyses, using voxel-based morphometry with age as a covariate, revealed significantly greater gray matter density in the right DLPFC of healthy participants relative to both Veterans ($p=0.05$, FWE corrected) and retired NFL players ($p=0.05$, FWE corrected). Findings also indicated significantly greater gray matter density in the left insula of healthy participants relative to Veterans ($p=0.05$, FWE corrected). Volume analyses, using age and total intracranial volume as covariates, revealed a trend level ($p=0.059$) difference in subcortical gray matter volume between the healthy participants and Veterans as well as the retired NFL players. Future volume analyses will focus on subcortical regions of interest. These findings suggest that Veterans and retired NFL players incurring mTBI and/or MHD may incur morphometric alterations in the DLPFC and insula: regions implicated in working memory, executive function and anxiety.

Disclosures: **A.A. Herrold:** None. **B.C. Harton:** None. **X. Wang:** None. **T. Parrish:** None. **Y. Chen:** None. **J.L. Reilly:** None. **H.C. Breiter:** None. **T.L.B. Pape:** None.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 44.07/D10

Topic: C.10. Trauma

Support: S.C. Holds a Career Award at the Scientific Interface from the Burroughs-Wellcome Fund

Washington University URSA Program

Title: Disambiguating structural and non-structural coma using network reachability analysis

Authors: M. KAFASHAN[1], S. RYU[2], D. ROBERTS[4], L. EISENMAN[4], T. T. KUMMER[4], *S. CHING[1,3];
[1]Electrical and Systems Engin., Washington Univ. In St. Louis, Saint Louis, MO; [3]Div. of Biol. and Biomed. Sci., [2]Washington Univ. in St. Louis, St. Louis, MO; [4]Neurol., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Coma is a state of severe cognitive impairment following brain injury. Although strictly pathological, a better understanding of coma could shed light on numerous aspects of

normal brain function. In particular, an analysis of how the lability of neural circuits - their overall dynamic range with respect to afferent excitation - is altered by brain injury could help define the underpinnings of normal cognitive functions in the brain. We report an analysis of the neural dynamics of coma using a new approach called 'Network Reachability Analysis (NetRA).' In this analysis scheme, a generative, dynamical, systems-based model for neural activity is used to fit observed EEG activity. A systems-theoretic reachability analysis is then performed on the model in order to quantify the effective dynamic range of the model. In essence, this analysis quantifies the extent to which the neural dynamics are labile with respect to ongoing recurrent activity. We proceeded to use NetRA to study EEG recordings from a cohort of 26 coma patients separated into two well-delineated coma variants: (i) coma arising due to focal injuries (structural coma) and (ii) diffuse, widespread injuries (non-structural coma). In conjunction with NetRA, we performed rigorous spectral and time-series analysis of these EEG data. We found that classical spectral analysis (e.g., band-limited EEG power) was not sufficient to disambiguate the two variants. Time-series analysis based on signal entropy did carry more discriminative power, with structural comas exhibiting more entropic variability overall (a surrogate for complexity). While useful as a discriminator, the highly statistical nature of these analyses did not allow for straightforward neurophysiological interpretations. In contrast, metrics derived from NetRA reliably (86% accuracy) disambiguated the two coma types and indicated that structural comas exhibited significantly more labile dynamics overall. Our results indicate that the dynamic range of neural activity in structural coma is generally larger than in non-structural coma, suggesting agreement with the intuitive interpretation that more circuits remain unaltered in the former condition. Importantly, this conclusion could not be made with conventional spectral analysis alone. Further, our findings show that mechanistic inferences regarding brain activity can be obtained from dynamical-systems based analyses. These inferences correlate with clinically-defined coma variants, and could be useful for clinical decision making and prognostication following brain injury.

Disclosures: **M. Kafashan:** None. **S. Ryu:** None. **D. Roberts:** None. **L. Eisenman:** None. **T.T. Kummer:** None. **S. Ching:** None.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 44.08/D11

Topic: C.10. Trauma

Support: CDMRP – MRMC Contract W81XWH-14-C-0139

Disclaimer: Any opinions, views, or assertions expressed are solely those of the authors and do not necessarily represent those of NICoE, WRNNMC, CNRM, the USUHS, the DoD, Department of Army/Navy/Air Force, or the U.S. Government

Title: The computer assisted rehabilitation environment as a tool for differentiating traumatic brain injury from post-traumatic stress disorder: a retrospective analysis of three virtual environments

Authors: *M. M. ONAKOMAIYA[1,2], S. E. KRUGER[1], K. B. HIGHLAND[3,4,5], M. J. ROY[3,4]; [1]Natl. Intrepid Ctr. of Excellence (NICoE), Walter Reed Natl. Military Med. Ctr., Bethesda, MD; [2]Cherokee Nation Technol. Solutions, Catoosa, OK; [3]Dept. of Med., [4]Ctr. for Neurosci. and Regenerative Med., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [5]Henry M. Jackson Fndn., Bethesda, MD

Abstract: Mild traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) are common, and frequently associated with functional impairment in service members (SMs) returning from the conflicts in Afghanistan and Iraq. TBI and PTSD are frequently comorbid; one study found that 44% of returning SMs with mTBI met criteria for PTSD. Presenting symptoms markedly overlap and standard neuropsychological assessments and neuroimaging techniques have been ineffective in discriminating between the two conditions. This has prompted efforts to improve clinical assessments to ensure that SMs receive accurate diagnoses, and appropriate and effective treatment. The Computer Assisted Rehabilitation Environment (CAREN) is a dynamic system that incorporates multi-planar motion within interactive virtual environments (VEs). The immersive and flexible VEs allow clinicians to integrate advanced technology in the assessment and rehabilitation of injured SMs. Thus, the objective of this study was to assess whether physical performance in CAREN tasks varies according to diagnosis. Data was obtained by independent, retrospective review of clinical notes and objective outcomes from 191 patients with TBI (186 Male) assessed in the CAREN from 2010-2015. Patients were sorted into two categories based on diagnoses in their medical history: TBI alone or comorbid TBI-PTSD. Three preliminary VE tasks were used to acclimatize patients: 1) Balance Balls (BB): weight-shifting on a static platform (time); 2) Balance Cubes (BC): step-shifting with and without platform motion (time); and 3) Continuous Road (CR): ambulation (self-selected speed). T-tests were used to determine mean differences between diagnostic categories in VE task performance. A binary logistic regression was used to examine whether VE task performance could predict diagnostic category. T-tests revealed that patients with comorbid TBI-PTSD had significantly slower self-selected speeds on the CR task ($p = 0.036$) and spent marginally more time on the BC task ($p = 0.05$) than patients with TBI alone. In addition, a logistic regression showed that the CR task significantly predicted diagnostic category ($\beta = -0.607$; $p = 0.031$; OR 0.55, 95% CI [0.31, 0.95]), such that a 1 mph decrease in speed was associated with a 55% greater likelihood of having comorbid PTSD. In a population of SMs with TBI, self-selected speed on the CAREN effectively distinguished those with comorbid PTSD, as they were significantly more likely to choose a slower speed than patients with TBI alone. These results

portray the potential of the CAREN as a novel assessment tool for differentiating patients with a dual diagnosis from patients with TBI alone.

Disclosures: **M.M. Onakomaiya:** None. **S.E. Kruger:** None. **K.B. Highland:** None. **M.J. Roy:** None.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 44.09/D12

Topic: C.10. Trauma

Support: CIHR to DE

Title: EEG correlates of enduring pscho-affective alterations in athletes with a history of concussion

Authors: *R. MOORE, W. SAUVE, D. ELLEMBERG;
Univ. of Montreal, Montreal, QC, Canada

Abstract: Understanding the neuropathological underpinnings of sports-related concussion is critical for aiding the diagnosis, prognosis, and remediation of concussive injuries. Although electro-encephalographic (EEG) methods have proved invaluable for detailing the neuro-motor and neuro-cognitive outcomes of concussion, none have ventured to implement these methods in order to delineate the psycho-affective outcomes of sports-related concussion. Accordingly, the primary aim of this study was to evaluate the relation of electroencephalographic activity in collegiate athletes to measures of psycho-affective function. Resting electro-encephalograms (EEG) and measures of mood and affect, including the Beck Depression Inventory-II (BDI-II) and Profile of Mood States (POMS) were collected in 81 male athletes (45 concussion history; 36 controls). Although athletes with a history of concussion were 9+ months from injury and "free of concussion symptoms", they exhibited alterations in frontal-alpha power and asymmetry, as well as frontal-beta asymmetry (p's< .05). Athletes with a history of concussion also exhibited alterations in frontal-delta power (p's<. 05). Correlational analyses revealed that frontal alterations in alpha and beta asymmetry were related to self-reported depression, anxiety, and anger, respectively ($r2's \geq .26$, $p's \leq .04$). The current study demonstrates that "asymptomatic" athletes who are presumed to be healed may still exhibit neural activity associated with increased levels of depression, anxiety and anger, and that EEG may serve as a sensitive for clinicians to identify and track concussion-related alterations in psycho-affective

states. Thus, the current study adds scientifically novel and clinically relevant information regarding an important, but poorly understood aspect of concussion.

Disclosures: **R. Moore:** None. **W. Sauve:** None. **D. Ellemberg:** None.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 44.10/D13

Topic: C.10. Trauma

Title: The effect of claustrum lesions on human consciousness and recovery of function

Authors: ***A. CHAU**[1], A. M. SALAZAR[2], F. KRUEGER[3], I. CRISTOFORI[4,1], J. GRAFMAN[4,1];
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Abstract: The claustrum is a thin sheet of grey matter located between the putamen and insula and has extensive reciprocal projections, connecting the claustrum to numerous anterior and posterior cortical and subcortical regions, such as the prefrontal cortex, primary sensory cortices, thalamus, and reticular formation. Damage to the claustrum could impact multiple neural networks. Given its structure and connectivity, the claustrum is postulated to play a role in coordinating a set of diverse brain functions. For example, Crick and Koch proposed that the claustrum plays a crucial role in consciousness. Given there are few human studies investigating this claim, we examined the effects of claustrum lesions on consciousness in 171 combat veterans with focal penetrating traumatic brain injuries. Additionally, we studied the effects of claustrum lesions and loss of consciousness on long-term cognitive abilities 30 years post-injury. Claustrum damage was predominantly associated with the duration, but not frequency, of loss of consciousness. Thus, the claustrum may have an important role in regaining, but not maintaining, consciousness. Total brain volume loss, but not claustrum lesions nor loss of consciousness, was associated with long-term recovery of neurobehavioral functions. Disruption to a greater number of functional networks, characterized by increasing total brain volume loss, may lead to a greater probability of loss of consciousness and poorer long-term cognitive outcomes. Our study is the largest study to date to investigate the effects of claustrum lesions on consciousness in humans. Our findings constrain the current understanding of the neurobehavioral functions of the claustrum and its' role in maintaining and regaining consciousness.

Disclosures: **A. Chau:** None. **A.M. Salazar:** None. **F. Krueger:** None. **I. Cristofori:** None. **J. Grafman:** None.

Poster

044. Traumatic Brain Injury: Human Studies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 44.11/D14

Topic: C.10. Trauma

Title: Mapping white matter changes in the corpus callosum following pediatric mild traumatic brain injury

Authors: ***S. WILCOX**[1], P. SERRANO[1], M. O'BRIEN[2], L. BECERRA[1], D. BORSOOK[1]; [1]Dept. of Anesthesiol., P.A.I.N Group, Waltham, MA; [2]Boston Children's Hosp., Division of Sport Medicine, MA

Abstract: Pediatric mild traumatic brain injury (mTBI) is the most prevalent neurological insult in children and is associated with both acute and chronic neurobehavioral sequelae, including headaches. White matter tracts are highly vulnerable to damage from impact-acceleration forces of TBI, with mTBI being characterized by evidence of traumatic axonal injury as assessed by diffusion tensor imaging. In particular, axons traversing the corpus callosum are particularly vulnerable to TBI. In this study we examined white matter integrity of the corpus callosum (CC) and specific inter-hemispheric tracts in adolescents at 3-12 months post-mTBI, compared to age-matched healthy controls. Group differences in diffusion parameters in the corpus callosum, as markers of altered white matter microstructure, were analyzed using tract based spatial statistics (TBSS). In addition, individual inter-hemispheric tracts were identified using probabilistic tractography. TBSS revealed that mTBI subjects displayed increased fractional anisotropy in the splenium and body, increased mean diffusivity in the splenium, decreased radial diffusivity in the body and increased radial diffusivity in the splenium, and increased axonal diffusivity throughout the entire CC (splenium, body and genu), compared to controls. These alterations in the corpus callosum's microstructural integrity may be part of the underlying pathomechanisms in post-concussive symptoms, such as headache. As such, diffusion parameters may serve a future clinical use as neuroanatomical indicators for early identification of children at risk of persistent post-concussive symptoms.

Disclosures: **S. Wilcox:** None. **P. Serrano:** None. **M. O'Brien:** None. **L. Becerra:** None. **D. Borsook:** None.

Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 45.01/D15

Topic: C.10. Trauma

Support: This study was supported by NIH/NINDS R01NS082308 (Loane).

CONACYT scholarship 249772/389071(DMAC)

Title: Microglia activation phenotypes and their modulation following experimental TBI

Authors: *A. KUMAR[1], J. BARRETT[1], D.-M. ALVAREZ-CRODA[1,2], B. STOICA[1], F. TCHANTCHOU[1], A. FADEN[1], D. LOANE[1];
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Abstract: Microglia can be polarized towards either an M1-like/classical or M2-like/alternative activation status in response to injury. These phenotypes can mediate neuroinflammation or promote tissue repair, respectively. Activation of NADPH oxidase (NOX2/gp91phox) is an important mechanism involved in pro-inflammatory signaling in microglia. We reported that NOX2 is chronically expressed in M1-like microglia in the peri-lesional area 1 year following controlled cortical impact (CCI) in mice. Here we compared wild-type (WT; gp91phox+/+) and NOX2-deficient (NOX2-KO; gp91phox-/-) mice to investigate the role of NOX2 in posttraumatic microglial polarization. Three-month old WT or NOX2-KO male mice were subjected to CCI (6m/sec, 2mm depth), and cohorts were followed for 1- 28d post-injury. M1-/M2-like polarization was analyzed by qPCR, flow cytometry, Western blot, and immunohistochemistry. Neurogenesis was assessed using doublecortin (DCX) immunohistochemistry; motor recovery and histology were assessed using a beam walk test and stereological methods. In WT TBI mice, NOX2 was expressed in reactive microglia (CD68+/Clic1+) in peri-lesional cortex through 28d; NOX2-KO significantly reduced CD68/Clic1 expression at 3 and 7d post-injury. Flow cytometry analysis of isolated microglia/macrophages revealed that IL-4Rα and its downstream signaling pathway (STAT6, JAK3) were significantly increased in CD45high microglia/macrophages of NOX2-KO TBI mice compared to WT controls. M2-like polarization (Arg1, Ym1, TGFβ) was increased at 3d post-injury in NOX2-KO, with effects sustained through 21d. There was significant reduction M1-like polarized microglia/macrophages (IL-1β, TNFα, IL-12, iNOS, CD16/32) in NOX2-KO TBI mice. M1/M2-like changes were associated with increased numbers of DCX-positive cells

in the SVZ, striatum and peri-lesional cortex, indicating increased neurogenesis. NOX2-KO TBI mice also showed significantly improved motor function and reduced cortical neurodegeneration at 21d. Thus, after TBI NOX2-KO mice exhibit enhanced IL-4Rα-mediated signaling, greater M2-like repolarization, and reduced neurodegeneration. Moreover, altering the M1-/M2-like balance appears to support increased neurogenesis. These data indicate that NOX2 drives the M1-like polarization of microglia/macrophages after TBI, and that inhibiting this pathway limits tissue damage and may promote tissue repair.

Disclosures: **A. Kumar:** None. **J. Barrett:** None. **D. Alvarez-Croda:** None. **B. Stoica:** None. **F. Tchantchou:** None. **A. Faden:** None. **D. Loane:** None.

Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.10. Trauma

Support: NIH AG030205

ADDF

Title: New developments in calcium-channel targeted therapeutics in AD: Preventing pathology from cellular to network levels

Authors: ***G. E. STUTZMANN**[1], M. GARSTKA[2], N. KAPECKI[2], E. HILL[3], S. CHAKROBORTY[2], C. A. BRIGGS[2], A. GILMAN-SACHS[4], K. BEAMAN[4], W. FROST[3]; [2]Neurosci., [3]Cell Biol. and Anat., [4]Microbiology and Immunol., [1]Rosalind Franklin Univ. /Chicago Med. Sch., North Chicago, IL

Abstract: AD displays multiple seemingly unrelated phenotypes whose underlying causes are poorly understood, rendering the disease very difficult to treat. One strategy to improve upon the disappointing therapeutic outcomes is to target more upstream disease mechanisms, such as abnormalities in ryanodine receptor (RyR) calcium signaling. Here we describe more detailed and broader evidence of the contribution of aberrant RyR-calcium signaling to the complex range of AD-associated pathology, and further validate this channel as a drug target. Particularly notable were alterations in properties of key acidic and protein-regulating organelles. Normally, maladaptive proteins are recycled through authophagosome-lysosome mediated complexes; lysosomes require proper vacuolar ATPase proton pump (vATPase) function to maintain the

acidic pH necessary to break down cargo transported from autophagosomes. Without proper vATPase composition, autophagosomes, damaged organelles, and mis-aggregated proteins such as amyloid and tau can accumulate. Using immunoassays and confocal microscopy, we demonstrate a marked reduction in V1B2 vATPase subunit levels in cortex and hippocampus of young 3xTg-AD mice, suggesting an impairment in proton pump capacity and pH maintenance. In parallel, there were significant increases in mature autophagosomes in the AD mice, indicative of accumulated mis-aggregated proteins. Notably, sub-chronic treatment with RyR-stabilizing compounds such as dantrolene restored normal V1B2 and autophagosome levels in AD mice; amyloid and phospho-tau levels were also reduced with this treatment. This indicates that increased RyR-calcium signaling is impinging upon critical organelles and affecting protein handling early in AD. At more global levels, we also reveal blunted hippocampal network propagation in presymptomatic AD mice which occurs in parallel with exaggerated synaptically-evoked calcium signals within CA1 dendritic spines with network activation. Using 2-photon calcium imaging to study localized cellular deficits, and voltage-sensitive dyes with CMOS-based imaging of hippocampal slices, we have identified novel local, circuit and network level deficits which may ultimately drive the later cognitive deficits in AD.

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Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

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Topic: C.10. Trauma

Support: R01 NS062097, R01 NS058710, R01 NS085568

Title: Intranasally delivered Wnt-3a enhances therapeutic effects of transplanted iPS cell-derived neural progenitor cells and increased endogenous regenerative activities after traumatic brain injury

Authors: *Z. WEI, X. GU, T. C. DEVEAU, J. LEE, M. M. WINTER, S. YU, L. WEI; Anesthesiology/Neurology, Emory Univ., Atlanta, GA

Abstract: Neonatal brain injury is a significant cause of mortality and long-term neurological deficits in infants and children. Studies to date using stem cell-derived neural progenitors have

shown great promise as a regenerative treatment against brain injury such as traumatic brain injury (TBI). The survival, migration and neuronal differentiation of these cells after transplantation, however, are poorly understood and need further improvements. Moreover, therapeutic outcomes from preclinical and clinical trials have been inconsistent and sometimes controversial. Although transplanted cells such as embryonic/induced pluripotent stem cell-derived neural progenitors (ES/iPS-NPCs), bone marrow stem cells (BMSCs) and neural stem cells (NSCs) have shown therapeutic benefits, the ability of these cells for sufficient morphological and functional recovery after brain injury has been unsatisfactory. In the current investigation, we tested the hypothesis that a strategy of combining transplanted cells with enhanced endogenous regenerative mechanism after TBI in the post-natal brain. We show in iPS-NPC cultures that recombinant Wnt-3a protein significantly increased cell survival, migration and neuronal differentiation of these cells. In P14 rats subjected to a closed impact insult, activating Wnt canonical pathway was enforced by intranasally-delivered Wnt-3a at 1 to 7 days after TBI. The iPS-NPCs were intranasally delivered at 3 and 7 days after TBI. At 14 days after TBI, the Wnt-3a plus iPS-NPC group showed the greatest increase of BDNF level in peri-contusion regions compared to both Wnt-3a alone group and iPS-NPC alone group. The Wnt-3a plus iPS-NPC treatment increased BrdU+NeuN+ cells and the level of myelin basic proteins (MBP). Behavioral tests and home cage monitoring showed significantly better improvements in sensory and motor function recovery in the Wnt-3a plus iPS-NPC group compared to both Wnt-3a group and iPS-NPC group. Our data highlight this novel combination method to enhance the cell-based neurorestorative therapy after neonatal TBI and other brain injuries.

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Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.10. Trauma

Support: NIH NS050465

NIH NS056413

Title: Boosting the power of exercise for brain trauma recovery using the capacity of a flavonoid derivative to activate BDNF-TrkB signaling

Authors: *F. GOMEZ-PINILLA[1,2], R. AGRAWAL[1], Y. ZHUANG[2], Z. YING[1], F. HONG[1]; [1]Integrative Biol. and Physiol., [2]Dept. Neurosurg., UCLA, Los Angeles, CA

Abstract: Traumatic brain injury (TBI) imposes a state of brain vulnerability in which neurons perform at a suboptimal level such that exposure to tasks involving activity can further compromise neuronal function. This period of neuronal vulnerability is particularly critical for patients engaging in physical rehabilitation programs or TBI encountered in sport practice. Although exercise has the intrinsic aptitude to counteract the effects of TBI, its application during the acute period of TBI may not be optimal. We studied the power of 7,8-dihydroxyflavone (7,8-DHF) to boost the effects of exercise on functional recovery after TBI. 7,8-DHF is a derivative of the flavonoid family abundant in fruits and vegetables, and a BDNF agonist that crosses the blood brain barrier. BDNF is one of the most influential molecules for brain function, which in addition to protecting neurons against a variety of insults, it has the unique capacity to counteract psychiatric like disorders within the spectrum of TBI pathology. The 7,8-DHF (5 mg/kg, ip) and/or exercise was administered daily in animals subjected to the fluid percussion injury (FPI). TBI reduced the BDNF-TrkB signaling and elements associated with regulation of energy homeostasis such as AMPK phosphorylation, cytochrome c oxidase II (COII) and regulators of mitochondrial biogenesis such as PGC-1α. These changes were concurrent with reductions in memory function observed in Barnes maze. Treatment with 7,8-DHF or exercise ameliorated reductions in the molecular systems under study, and more importantly, 7,8-DHF boosted the action of exercise after TBI. *In vitro* studies showed that 7,8-DHF (200 and 400 nM) upregulates the levels of biogenesis activator PGC-1α, and CREB phosphorylation, suggesting that activation of BDNF-TrkB signaling is pivotal for synaptic plasticity and energy metabolism. The treatment with 7,8-DHF (200 nM) also elevated the mitochondrial respiratory capacity, measured by Extracellular Flux analyzer, which emphasizes the role of BDNF-TrkB signaling as mitochondrial bioenergetics stimulator. This study highlights the power of 7,8-DHF to speed functional recovery, and to reduce the period of convalescence following TBI. This study emphasizes BDNF-TrkB signaling for supporting functional recovery following TBI engaging the interplay between mitochondrial function and synaptic plasticity.

Disclosures: F. Gomez-Pinilla: None. **R. Agrawal:** None. **Y. Zhuang:** None. **Z. Ying:** None. **F. Hong:** None.

Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 45.05/D19

Topic: C.10. Trauma

Support: The Moody Foundation

The Cullen Trust

The Alzheimer's Drug Discovery Foundation

The Mitchell Center for Neurodegenerative Diseases

Title: Tau oligomers and traumatic brain injury: toxicity and potential drug targets

Authors: *B. E. HAWKINS[1], J. GERSON[2], U. SENGUPTA[3], D. CASTILLO-CARRANZA[3], D. PROUGH[4], D. DEWITT[4], R. KAYED[3];
[2]Neurosci. and Cell Biol., [3]Neurol., [4]Anesthesiol., [1]Univ. of Texas Med. Br., Galveston, TX

Abstract: Traumatic brain injury (TBI) is a chronic condition and may predispose individuals to develop an Alzheimer's disease-like dementia at an early age. Although the aggregation and accumulation of tau-based neurofibrillary tangles have traditionally been recognized as indicators of Alzheimer's, soluble tau oligomers are now thought to play a role in spreading the pathology. Tau oligomers exert their effects by causing toxicity in cells where they are present, but their soluble nature also allows them to spread from affected to unaffected brain regions. To demonstrate the ability of tau oligomers to spread and cause negative effects, we isolated tau oligomers from rodent brain homogenates that had received either an impact or blast-induced TBI and then injected those tau oligomers into htau mice that consequently developed spatial learning and memory deficits. Targeting extracellular tau oligomers may effectively halt the disease process. Passive immunotherapy is a promising mode of treatment against neurodegenerative disease because antibody doses can be controlled and specifically targeted to toxic species without impacting functional proteins. Anti-tau oligomer specific monoclonal antibodies (TOMA) is unique in that only recognizes oligomeric tau, a toxic form of the microtubule protein tau, while leaving normal, functional tau protein intact. We have demonstrated an increase in tau oligomers after fluid percussion injury in nontransgenic animals and attempted to block this increase by administering a single treatment with TOMA 24hrs after injury. We examined neuronal injury (using FluoroJade), vascular reactivity and tau pathology and found a reduction in FJ+ neurons in the TOMA-treated animals, suggesting a possible therapeutic effect. We conclude from these data that tau oligomers are toxic following TBI and that TOMA appears to have a beneficial effect following injury, most likely by binding to the toxic tau oligomers that accumulate after fluid percussion TBI. These studies were completed as part of an interdisciplinary research team funded by The Moody Project for Translational Traumatic Brain Injury Research.

Disclosures: **B.E. Hawkins:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **J. Gerson:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **U. Sengupta:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **D. Castillo-Carranza:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **D. Prough:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **D. DeWitt:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch. **R. Kayed:** A. Employment/Salary (full or part-time):; University of Texas Medical Branch.

Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 45.06/D20

Topic: C.10. Trauma

Support: NIH Grant

Title: Ephrin-B3 restricts endogenous neural stem cell migration in the peri-lesional region following traumatic brain injury

Authors: ***K. J. DIXON**[1], E. J. PEREZ[2], J. MIER[2], A. TURBIC[4], A. M. TURNLEY[4], D. J. LIEBL[3];
[1]Virginia Commonwealth Univ., Richmond, VA; [2]Univ. of Miami, Miami, VA; [3]Univ. of Miami, Miami, FL; [4]Univ. of Melbourne, Melbourne, Australia

Abstract: Traumatic brain injury induces a robust induction of SVZ-derived neural stem/progenitor cell (NSPC) proliferation and differentiation. These NSPCs then migrate into the peri-lesional region where they initially provide neuroprotection from the mechanical insult and eventually differentiate into mature neurons. However, the factors regulating this migratory process have not been assessed. Eph receptors and their ephrin ligands negatively regulate SVZ-derived NSPC proliferation and survival, and are known regulators of cell and axonal migration. Ephrin-B3 regulates neural crest cell migration and was recently implicated in NSPC migration along the naïve RMS, therefore we sought to investigate the role of ephrin-B3 in NSPC migration, before and after TBI. We stereologically counted the number of NSPC migrating under naïve and TBI conditions using a model of cannula cortical injury infusing soluble ephrin-B3/Fc, in both wildtype and ephrin-B3-/- mice. Data from ephrin-B3-/- mice and infusion studies suggest ephrin-B3 restricts ectopic NSPC migration before and after TBI, and their mode of migration differs along a rostro-caudal gradient. In the rostral in the corpus callosum overlying

the RMS NSPCs appear to use chain migration in close proximity to the vasculature, while in the caudal corpus callosum and peri-lesional region the NSPCs migrate as single, dissociated cells seemingly independent of the vasculature. Additionally, acutely after injury some of the existing NSPCs were re-routed from the RMS to migrate to the peri-lesional region. To identify a direct or indirect effect of ephrin-B3 on the NSPCs we cultured SVZ explants and quantitated NSPC migration in the presence and absence of soluble ephrin-B3/Fc. The data shows unclustered (antagonist) ephrin-B3/Fc promotes chain migration, while clustered (agonist) ephrin-B3/Fc enhances dissociated single cell migration. Therefore, following cortical injury NSPCs in the SVZ migrate either along the RMS or re-routed towards the peri-lesional region, with extracellular ephrin-B3 restricting their migration. This data sheds light on the factors regulating NSPC migration before and after TBI and opens the window for future therapeutic interventions.

Disclosures: K.J. Dixon: None. **E.J. Perez:** None. **J. Mier:** None. **A. Turbic:** None. **A.M. Turnley:** None. **D.J. Liebl:** None.

Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

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Support: NFL Charities Foundation

Indiana Department of Health Spinal Cord and Brain Injury Fund

Mizzou Advantage

University of Missouri Department of Pathology & Anatomical Sciences Research Fund

Title: Metabolite of gelatinase inhibitor prodrug attenuates brain damage and improves sensorimotor functions in a mouse model of severe traumatic brain injury

Authors: *R. NIZAM[1,2], Z. CHEN[1,2], B. TOMLINSON[1,2,3], O. HADASS[1,2,3], W. SONG[6], M. IKEJIRI[6], M. JUÁREZ[1,2], S. CHEN[1,2,4,5], J. CUI[1,2,4,7], S. MOBASHERY[6], M. CHANG[6], Z. GU[1,2,4,7];
[1]Pathology and Anatom. Sci., [2]Ctr. for Translational Neurosci., [3]MS in Pathology Program, [4]Ctr. for Botanical Interaction Studies, [5]Interdisciplinary Neurosci. Program, Univ. of Missouri - Columbia, Columbia, MO; [6]Chem. and Biochem., Univ. of Notre Dame, Notre Dame, IN; [7]Harry S. Truman Mem. Veterans' Hosp., Columbia, MO

Abstract: Traumatic brain injury (TBI) is a prevalent condition affecting 1.7 million individuals in the United States. After the initial injury, biochemical processes often lead to a second stage of brain injury that is considered the cause of many neurological dysfunctions. Biochemical, metabolic and cellular changes observed during this secondary injury are often associated with disruption of the blood-brain barrier (BBB), inflammatory responses and infiltration of blood-derived macrophages, edema, and cell death. Studies in our laboratory and others suggest that irregular signaling events seen after a TBI can lead to activation of endopeptidase enzymes called matrix metalloproteinases (MMPs) which can digest the extracellular matrix and tight junction molecules and cause axonal degeneration, resulting in edema, hemorrhage and brain damage. Among the 23 known human MMPs, MMP-9 (gelatinase B) in particular has been correlated with neuroinflammation and white matter damage in the brain. ND-478 is a water-soluble gelatinase inhibitor prodrug which is hydrolyzed into the active MMP-9 inhibitor ND-322. ND-322 is then *N*-acetylated to ND-364 in the brain and liver. Both ND-322 and ND-364 are capable of crossing the BBB, but only ND-364 achieves therapeutic concentrations in the brain. Thus, we chose to administrate ND-364 at a dose of 28 mg/kg. In this study, we performed a controlled cortical impact TBI model in mice, and subsequently treated them with ND-364 or vehicle injected at 2 and 4 hours after the surgery followed by daily treatment for the next 6 days. Three treatment groups were tested with different routes of administration: (**1**) subcutaneous injections only; (**2**) intraperitoneal injection for the first dose, followed by subcutaneous ones; and (**3**) intraperitoneal injections only. We observed neurological behaviors using beam-walking and a Simple Neuroassessment of Asymmetric imPairment (SNAP) test to evaluate motor, sensory and reflex abilities before surgery, and at 3 and 7 days post-surgery. We then dissected the brains at 7 days post-surgery for coronal sectioning, and stained brain sections with cresyl violet. Microscopic whole-slide image (WSI) analysis revealed that ND-364 was efficacious and significantly reduced cortical lesion percentage in all groups compared to vehicle. ND-364 significantly improved mouse behavior outcomes in groups **1** and **2**, but not in group **3**. In summary, these findings indicate that selective inhibition of MMP-9 by a second-generation thiirane gelatinase inhibitor is a promising therapy.

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Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.10. Trauma

Support: DoD W81XWH-13-2-0091; Dept. of Veterans Affairs

Title: Veliparib suppresses microglial activation after brain trauma in rats and pigs

Authors: *K. A. IRVINE, J. XU, R. K. BISHOP, P. SINGH, A. SONDAG, K. HAMEL, V. COPPES, D. J. KAPFHAMER, S. WON, S. PANTER, R. A. SWANSON;
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Abstract: Brain trauma induces an innate immune response involving the activation of resident microglial and the infiltration of circulating immune cells. These cells release cytotoxic cytokines, proteases, and reactive oxygen species which may exacerbate injury and impair recovery. Inhibitors of poly(ADP-ribose) polymerase-1 (PARP-1) are also known to effectively suppress the innate immune response. The mechanism of this effect is not well understood, but likely involves blocking the transcriptional activities of the pro-inflammatory transcription factors NF-κB and/or AP1. Veliparib is an extremely potent, orally active inhibitor of (PARP-1) that is currently in clinical trials for certain types of cancers. In this study we are evaluating the effects of veliparib on outcomes after cortical controlled impact (CCI) in rats and pigs. Veliparib was given intraperitoneally to rats or orally to pigs beginning 2 hours after CCI at doses ranging from 0 to 9 mg/kg. Doses of above 1 mg/kg were found to markedly suppress microglial activation in both rats and pigs, as assessed by Iba-1 expression and microglial morphology. RT-PCR measures of gene expression in microglia isolated from rat brains, at times ranging from 6 hours to 14 days after CCI, showed that veliparib also attenuated the increase in matrix metalloproteinase-9 and NADPH oxidase-2 that otherwise occurred in peri-lesional microglia after CCI. Ongoing studies are evaluating the histological and behavioral correlates of this treatment approach, and the efficacy of veliparib treatment initiated at longer intervals after brain trauma.

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Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

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Topic: C.10. Trauma

Support: NIH NS059622

NIH NS050243

Title: Neuroprotection against traumatic brain injury through disrupting the interaction of nNOS with PSD95

Authors: ***W. QU**[1,2], N. LIU[1], X. WU[1], Y. WANG[1], Y. SUN[1], Y. LAI[3], A. SHEKHAR[1], X.-M. XU[1];
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Abstract: Traumatic brain injury (TBI) is the leading cause of death and disability in the most active population (<45 years of age) in the United States. Unfortunately, there have been no pharmaceutical interventions with proven efficacy to date. Here we propose that the glutamate-NMDAR-PSD95-nNOS cascade induces neural toxicity and mediates functional deficits following TBI. We hypothesize that ZL006, a small-molecule inhibitor of the nNOS-PSD95 interaction, may disrupt such detrimental cascade, resulting in neuroprotection and functional recoveries following TBI. In an *in vitro* model of primary cortical neuron culture, we examined whether ZL006 has an effect on glutamate-triggered excitotoxicity and neuronal death using LDH releasing and propidium iodide (PI) inclusion assays. *In vivo*, a moderate controlled cortical impact (CCI) injury to the right cerebral cortex was induced in the C57Bl/6 mouse followed by administration of ZL006 at two doses (5mg/Kg and 10mg/Kg) or saline at 30 min post-injury and daily thereafter up to 7 days. Behavioral outcomes were measured weekly from 2 to 28 days post-injury using composite neuroscore, adhesive removal test, rotarod, Morris water maze (WMM), direct contact social test, and social activity and novelty. Post-injury lesion volumes were quantified on tissue sections after Cresyl violet staining. Our result showed that exposure to glutamate *in vitro* induced neuronal loss and neurite disintegration. ZL006 treatments at both doses promoted a significantly reduction of neuronal cell death. ZL006 treatment also significantly decreased LDH release. *In vivo*, mice treated with ZL006 showed improved motor function (neuroscore and rotarod tests) as early as the first week post-injury. In the adhesive removal test, both contact and removal time in the ZL006-treated groups were significantly shorter than the vehicle group. Furthermore, ZL006-treated mice showed significant improvements in learning and memory (MWM test) in the strategy to find the hidden platform at 16 and 18 days as compared to the vehicle-treated group. Finally, ZL006 treatment attenuated cognitive impairment in direct contact social test and social activity and novelty. The observed functional improvements were closely associated with histological reduction of the lesion volume in the ZL006-treated groups. In conclusion, ZL006 protected primary cortical neurons from glutamate-induced excitotoxicity and cell death *in vitro*, reduced lesion volume, and improved sensorimotor, learning and memory, and cognitive recoveries in a mouse model of TBI. By targeting nNOS-PSD95, ZL006 may offer a new opportunity to treat patients with TBI.

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Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.10. Trauma

Support: NSFC 81471269

NSFC 81300998

Title: Suppression of Ca^{2+}-independent PLA2γ increased mitochondrial damage after *in vitro* traumatic brain injury

Authors: ***J. JI**[1,2], H. CHAO[3], H. CHEN[4], N. LIU[3];
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Abstract: Ca2+-independent PLA2γ (iPLA2γ) hydrolyze phospholipids at the sn-2 position , thereby releasing free fatty acids and a lysophospholipids. iPLA2γ also participate in membrane phospholipid metabolism and remodeling especially under oxidative stress. The location of iPLA2γ is on endoplasmic reticulum and mitochondria. This implies that iPLA2γ may participate the membrane phospholipid injury and repair when mitochondria under oxidative stress. And This mitochondrial damage is common in neurodegeneration and trauma brain injury. So we use (R)-BEL, a specific inhibitor of iPLA2γ , to clarify the role of iPLA2γ when under mitochondrial oxidative stress damage after stretch in primary cortical neurons. In the current report, we found that iPLA2γ inhibitor increase the damage of mechanical stretch. Cytochrome c is released into the cytoplasm and caspase3 apoptosis pathway is activated. Mechanical stretch damage the mitochondrial membrane potential, and iPLA2γ inhibitor aggravated the damage. The inhibition of iPLA2γ may increase the mitochondrial apoptosis and injury. Further more, MDA,4-HNE, products of lipid peroxidation, increase after stretch, and iPLA2γ inhibitor exacerbated the lipid peroxidation. And iPLA2γ inhibitor also increased stretch-induced ROS production by DCFH-DA staining. Interestingly after stretch iPLA2γ expression level did not change significantly, but iPLA2γ activity significant increased. Our result suggested that iPLA2γ is neuroprotective and iPLA2γ can be up-regulated to relieve the mitochondrial injuries. Further mechanism about the iPLA2γ after stretch needs to be clarified .

Disclosures: **J. Ji:** None. **H. Chao:** None. **H. Chen:** None. **N. Liu:** None.

Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 45.11/D25

Topic: C.10. Trauma

Support: NSF grant EPS-1003907

WV SURE award (A.R.C.)

Title: Implantable fibrin cylinders that recruit new cells into the striatum and cortex

Authors: ***A. CLARK**[1], L. E. HAGER[2], E. M. PRICE[2];
[2]Biol., [1]Marshall Univ., Huntington, WV

Abstract: Adult neural progenitor cells (NPCs) are constantly produced in the mammalian subventricular zone (SVZ), where they migrate along the rostral migratory stream (RMS) to the olfactory bulb. Our laboratory is interested in engineering fibrin cylinders containing a variety of factors that will, upon surgical implantation into rat brain, redirect SVZ-NPCs from their usual route into a new, typically non-neurogenic region. This has therapeutic possibilities for a number of diseases and disorders such as Parkinson's disease and traumatic brain injury. Such applications will require restoring functional neurons to both the striatum and the cortex. These are distinct regions of the brain, arising through different developmental mechanisms and containing different cell types. Due to these differences, it is reasonable to assume that restoring cells to these regions will require different factors for the striatum versus the cortex. We have recently used 3 mm long fibrin cylinders containing NGF and VEGF to recruit new neurons into the striatum of rats when implanted through the RMS and into the striatum. In order to minimize reactive astrocyte infiltration into the implant region, we administered dexamethasone for four days following the surgery. This significantly reduced the number of GFAP+ cells along the implant track. However, this procedure led to significant iatrogenic damage to the cortex since the implant apparatus had to pass through the cortex as it was being placed in the striatum. In order to prevent this damage to the cortex, we prepared 6 mm long VEGF/NGF cylinders which, upon implantation, spanned the entire distance from the surface of the brain to the striatum. This maneuver resulted in nearly complete restoration of the cortical material after 6 weeks. Although a significant number of new neurons were seen in the striatum, few new neurons were seen in the cortex despite the apparent healing of the region. We conclude that the factors (NGF and VEGF)

which were responsible for recruiting cells into the striatum were not capable of accomplishing the same result in the cortex. This suggests that the recruitment of new neurons into the cortical region requires different neurotrophic microenvironments than those required by the striatum. We then engineered cylinders with different factors in each half; the portion implanted in the striatum contained NGF and VEGF and the other half which passed through the cortex contained GDNF and bFGF. We anticipate that the ability to custom engineer these cylinders will enable investigators to create implantable microenvironments containing the appropriate factors for the region of interest.

Disclosures: A. Clark: None. **L.E. Hager:** None. **E.M. Price:** None.

Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

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Topic: C.10. Trauma

Support: NIH grant NS084823

NIH grant P30 GM103507

Title: Amelioration of traumatic brain injury-induced cerebrovascular hyper-permeability by adipose tissue derived stromal vascular fraction cells

Authors: *N. MURADASHVILI[1], R. TYAGI[1], J. DALE[2], R. L. BENTON[3], S. C. TYAGI[1], J. B. HOYING[2], D. LOMINADZE[1];
[1]Physiol. & Biophysics, [2]Cardiovasc. Innovation Inst., [3]Anatom. Sci. and Neurobio. & Kentucky Spinal Cord Injury Res. Ctr., Univ. of Louisville, Louisville, KY

Abstract: Traumatic brain injury (TBI) is accompanied with development of inflammation and cognitive dysfunction. It contributes to vasculo-neuronal disorders leading to blood brain barrier disruption linked to increased cerebrovascular permeability to blood proteins. The loss of memory may be associated with the enhanced formation of un-degradable protein complexes such as fibrinogen (Fg) and cellular prion protein (PrP^C) complex after TBI. It is suggested that impairment in endothelial cell properties can be a main cause of enhanced protein extravasation/, and the resultant vasculo-neuronal degeneration. We tested the hypothesis that the treatment of vascular endothelium by anti-inflammatory and reparative stromal vascular fraction (SVF) cells derived from adipose tissue can mitigate TBI-induced hyper-permeability and reduce memory

loss by decreasing Fg-PrP^C complex formation. Permeability of pial venules in pericontusional area was studied in C57BL/6J mice brain after mild TBI. The day after induction of TBI, mice were injected with SVF cells suspended in 100 l of phosphate buffered saline (PBS) or with PBS alone (control group) through the jugular vein. After 10 days, cerebrovascular permeability in these mice was assessed by measuring the extravascular accumulation of Alexa-Fluor 647-labeled bovine serum albumin using an intravital fluorescence microscope. The protein leakage to interstitium was analyzed by changes in the ratio of fluorescence intensity in the interstitium to that inside the respective vascular segment. Cerebrovascular protein leakage was lesser in mice infused with SVF cells (131 ± 3 %) compared to that in mice infused with PBS alone (186 ± 6 %). Depositions of Fg and PrP^C in pericontusional area were mitigated in mice treated with SVF cells. Novel object recognition test showed a tendency of reduction in memory loss in mice injected with SVF cells. These results suggest that TBI-induced increased cerebrovascular permeability can be reversed by SVF cells, which can restore impaired property of vascular endothelium or cerebral microvascular network in pericontusional area. Thus, our data indicate a novel therapeutic role of SVF cells in treatment of vasculo-neuronal dysfunction after TBI.

Disclosures: N. Muradashvili: None. **R. Tyagi:** None. **J. Dale:** None. **R.L. Benton:** None. **S.C. Tyagi:** None. **J.B. Hoying:** None. **D. Lominadze:** None.

Poster

045. Traumatic Brain Injury: Therapeutic Strategies I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 45.13/D27

Topic: C.10. Trauma

Support: TEVA Pharmaceutical Industries

Title: Effects of laquinimod on microglia and monocytes following traumatic brain injury

Authors: *A. KATSUMOTO[1], A. S. MIRANDA[2], O. BUTOVSKY[3], Z. FANEK[3], R. M. RANSOHOFF[1], B. T. LAMB[1];
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Abstract: Background: Laquinimod is an orally administered neuroimmunomdulator in development for the treatment of multiple sclerosis (MS) and Huntington's disease (HD). The current studies utilized a model of traumatic brain injury (TBI) to assess the effect laquinimod

has on TBI induced neuroinflammation including microglial activation and monocyte infiltration along with persistent inflammation and neurodegeneration. **Methods:** To test the hypothesis that laquinimod differentially effects microglia and monocytes to alter inflammation following TBI, $CX3CR1^{GFP/+}$ $CCR2^{RFP/+}$ mice were utilized to isolate microglia and infiltrating monocytes following TBI and examined via nanostring analysis for the levels of immune gene mRNAs. Mice were administered laquinimod or vehicle by oral gavage before and after the TBI and sacrificed 3 days after the injury for gene expression analyses. **Results:** In monocytes, laquinimod up-regulated genes related to phagocytosis and down-regulated toll-like receptor (TLR) signaling and adhesion molecules. Notably, we also found a significant elevation of $CD45^{+}CCR2^{+}Ly6C^{high}$ inflammatory monocytes in the brain 3 days following TBI by flow cytometry, which was reduced by laquinimod treatment ($p<0.05$). Furthermore, gene expression analyses demonstrated microglia up-regulate TLR-4 cascade and NFκB signaling and at the same time down-regulate inflammatory (IL-6 and IL-8) at 3 days following TBI. Laquinimod treatment promoted an anti-inflammatory gene expression profile within microglia and hierarchical clustering showed that laquinimod treatment attenuated TBI-induced microglial gene expression closer to the sham group. In addition, immunohistochemistry showed that axonal damage was lessened following laquinimod treatment. Finally, laquinimod restored inhibited neurogenesis and enlarged ventricles induced by TBI. **Conclusions:** The current results suggest that laquinimod acts on both monocytes and microglia following TBI to attenuate inflammation by both reducing the recruitment of inflammatory monocytes as well as promoting a more sham-like microglial phenotype that leads to an amelioration of neuronal-based alterations induced by TBI.

Disclosures: **A. Katsumoto:** 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.; TEVA Pharmaceutical Industries. **A.S. Miranda:** None. **O. Butovsky:** 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.; TEVA Pharmaceutical Industries. **Z. Fanek:** 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.; TEVA Pharmaceutical Industries. **R.M. Ransohoff:** None. **B.T. Lamb:** 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.; TEVA Pharmaceutical Industries.

Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 46.01/D28

Topic: C.10. Trauma

Support: PVA

Title: Rewiring of spinal respiratory neural network via cervical glutamatergic interneurons preserves respiratory function following chronic cervical spinal cord injury (cSCI)

Authors: *K. SATKUNENDRARAJAH, S. K. KARADIMAS, M. KHAZAEI, P. MERCADO, G. YAO, K. JACQUES-SMITH, M. G. FEHLINGS;
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Abstract: Chronic and progressive compression of the cervical spinal cord disrupts the respiratory neural network leading to ventilatory dysfunction. In contrast to the severe respiratory insufficiency that follows acute traumatic cSCI, progressive cSCI results in milder dysfunction. Here, using a unique mouse model of progressive cSCI at C4-C6 vertebral level we attempt to elucidate this phenomenon. First, to neurophysiologicaly assess the modifications of spinal respiratory network induced by the progressive cSCI, we monitored diaphragmatic respiratory motor output after a left C2 hemisection injury (C2Hx) in cSCI and non-cSCI mice. C2Hx immediately silenced the ipsilateral respiratory function in non-cSCI mice, while cSCI mice had preserved ipsilateral respiratory motor output. Interestingly, a progressive loss of phrenic motoneurons (PMNs) with concurrent increase in Vglut2 positive boutons onto the preserved PMNs was found. This data led us to postulate that spinal glutamatergic interneurons (INs) relay and mediate rewiring between the brain stem respiratory CPG and the spinal respiratory network that curtails respiratory deficits in cSCI. To confirm this, dual injection of Rabies-Glycoprotein (RG) and Rb-ΔG-blue and HSV-Syn-GFP into the rVRG of the brainstem of cSCI and non-cSCI Vglut2::cre;tdtomato mice revealed that the connectivity between brainstem nuclei providing respiratory drive and PMNs is increasingly relayed through spinal glutamatergic INs under the progressive and chronic cSCI. Finally, to specifically silence the cervical glutamatergic relay interneurons (INs), we injected VSVG-pLV-TRE::IVMb-RFP and VSVG-pLv-vGlut2::aMVI-mPlum into the rVRG nuclei and RABV-Flippase-Cerulean and RG into the diaphragm muscle of Hoxb8-cre :: rTA GFP mice at 8 weeks post-cSCI. Subsequent ivermectin administration after C2Hx in these cSCI mice silenced this specific population and prevented the manifestation of preserved ipsilateral diaphragmatic EMG activity. Together, our data show that prephrenic spinal glutamatergic INs play a vital role in maintaining descending excitatory glutamateric drive to PMNs and maintain respiratory function in chronic and progressive cSCI. Moreover, these novel insights into the neural control of breathing can direct translational research for respiratory recovery in cSCI.

Disclosures: **K. Satkunendrarajah:** None. **S.K. Karadimas:** None. **M. Khazaei:** None. **P. Mercado:** None. **G. Yao:** None. **K. Jacques-Smith:** None. **M.G. Fehlings:** None.

Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.10. Trauma

Support: NSERC Discovery Grant

British Columbia Medical Services Foundation

Title: Altered co-activation across the frequency spectrum of lower extremity muscles in individuals with incomplete spinal cord injury

Authors: ***S. S. LEE**[1], T. LAM[2], K. PAUHL[2], E. HARDER[3], J. M. WAKELING[3]; [1]Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL; [2]Sch. of Kinesiology, The Univ. of British Columbia, Vancouver, BC, Canada; [3]Biomed. Physiol. and Kinesiology, Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Co-activation of antagonistic muscles is prevalent in individuals with incomplete spinal cord injury (SCI) and contributes to mobility limitations. In addition to kinematics and kinetic measurements to evaluate gait function, electromyography (EMG) is often used to measure muscle activity. In this study, we use wavelet analysis to resolve EMG signals into frequency components that provide information beyond the standard timing and amplitude measures. There is evidence that muscle fiber composition is altered in individuals with SCI and also influences the spectral properties of the EMG signal. The purpose of this study was to investigate co-activation in the lower extremity muscles: rectus femoris (RF), biceps femoris (BF), medial gastrocnemius (MG), and tibialis anterior (TA) in individuals with SCI and healthy age-matched unimpaired individuals using wavelet techniques and establishing an EMG normalcy score using principal component analysis as an indication of muscle function. A second aim was to determine if this type of frequency analysis of EMG signals would be effective and sensitive in evaluating the influence of body weight supported treadmill training that used robot-applied resistance at the hip and knee joints. Our main findings demonstrate that the co-activations of the knee extensor-flexors, RF-BF, and ankle plantar- and dorsiflexors, MG-TA, were significantly different between the individuals with incomplete SCI and age-matched control subjects. There were significant differences in the correlation coefficient of the total

EMG intensity between the SCI group and unimpaired control group for stride and stance phase. The correlation spectra, which indicate co-activation across the frequency spectrum, were also significantly different between the SCI and control group, in particular for the ankle muscles. The EMG-normalcy score, that considered the recruitment of different motor unit types by analyzing the frequency component of the EMG signal, supported the correlation coefficient results and also detected a difference between the pre-and post training for the MG-TA pair for stride and stance. In addition, both differences in pre-and post training EMG normalcy score and correlation coefficient of total intensity were correlated with pre- and post-training clinical tests such as the 6 Minute Walk Test and 10 Meter Walk Test. Application of this EMG analysis to quantify and evaluate muscle dysfunction and coordination in SCI offers new insights into the fundamental mechanisms behind SCI impaired gait and into the effectiveness of rehabilitation treatments.

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Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

Location: Hall A

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Topic: C.10. Trauma

Support: NIH Grant NS081738

Title: Optical dissection of cortical plasticity during recovery from spinal cord injury

Authors: *E. R. HOLLIS, N. ISHIKO, C.-C. LU, A. HAIMOVICH, Y. ZOU;
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Abstract: The learning of skilled tasks in rodents and primates is dependent upon circuit plasticity of motor representations within the motor cortex. Changes in these topographic motor maps are known to occur after spinal cord injury in animal models as well as in patients, however it is unknown whether motor maps are affected by increasing the plasticity of corticospinal motor axon collaterals after injury. Here we utilize optogenetic stimulation to examine motor map changes over time after the conditional deletion of the repulsive Wnt morphogen receptor related to receptor tyrosine kinase (Ryk), which limits corticospinal plasticity after spinal cord injury. We found that increased corticospinal axon collateralization after injury corresponded with a stabilization of the expanded cortical motor representations from

above the level of the injury. Recovery of skilled forelimb function was enhanced by increased axon collateralization and subsequently returned to control levels following the elimination of enhanced collateralization through a secondary injury. Our findings that 1) motor map changes in rodents mirror those that occur after spinal cord injury in patients and 2) that the corticospinal motor circuit can incorporate induced axonal changes, indicate that the axon repulsive Wnt-Ryk signaling axis is an attractive therapeutic target following spinal cord injury.

Disclosures: E.R. Hollis: None. **N. Ishiko:** None. **C. Lu:** None. **A. Haimovich:** None. **Y. Zou:** None.

Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

Location: Hall A

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Topic: C.10. Trauma

Support: RGC GRF HKUST662011

RGC GRF HKUST16101414

Title: Pten deletion promotes regeneration of corticospinal tract axons one year after spinal cord injury

Authors: *K. LIU, S. ZHENG;
HKUST, Kowloon, Hong Kong

Abstract: Chronic spinal cord injury (SCI) is a formidable hurdle that prevents a large number of injured axons from crossing the lesion, particularly the corticospinal tract (CST). This study shows that Pten deletion in the adult mouse cortex enhances compensatory sprouting of uninjured CST axons. Furthermore, forced upregulation of mTOR initiated either one month or one year after injury promoted regeneration of CST axons. Our results indicate that both developmental and injury-induced mTOR down-regulation in corticospinal motor neurons can be reversed in adults. Modulating neuronal mTOR activity is a potential strategy for axon regeneration after chronic SCI.

Disclosures: K. Liu: None. **S. Zheng:** None.

Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

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Title: The role of RhoA in retrograde neuronal death and axon regeneration after spinal cord injury

Authors: *J. HU, G. ZHANG, W. RODEMER, M. SELZER;
Shiners Hosp. Pediatric Res. Ctr. (SHPRC), Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Paralysis following spinal cord injury (SCI) is due to failure of axonal regeneration. It is believed that axon growth is inhibited by the presence of several types of inhibitory molecules in central nervous system (CNS), including the chondroitin sulfate proteoglycans (CSPGs). This also is true in the lamprey, whose brainstem contains 18 pairs of individually identified reticulospinal neurons with heterogeneous axon regenerative abilities. The “bad-regenerating” neurons often experience a delayed form of retrograde cell death. These same bad-regenerators selectively express the CSPG receptors PTPsigma and LAR, suggesting that CSPGs may affect intracellular signaling via their receptors. There is evidence that after SCI, the small GTPase RhoA is an intracellular convergence point for signaling by several extracellular inhibitory molecules, including CSPGs. RhoA also has been implicated in local neuronal apoptosis after SCI. Thus we hypothesize that RhoA may be involved in the delayed retrograde neuronal death in lamprey brain after SCI. To test this, we cloned lamprey RhoA and found its mRNA was expressed in both neurons and glia. Levels of active RhoA (RhoA-GTP) increased after spinal cord transection (tx). Next we designed morpholinos to knockdown RhoA *in vivo*, which reduced expression of total RhoA in the spinal cord by ~20%. The knockdown effect was even more robust in individual axons (~40%). RhoA knockdown significantly reduced retrograde apoptosis signaling in identified neurons in lamprey brains post-tx, as indicated by Fluorescent Labeled Inhibitor of Caspases (FLICA) in brain wholemounts. The reduction of caspase activity in the brain by knockdown of RhoA began at 2 weeks and was still seen at 10 weeks. Moreover, by measuring the distance between individual axon tips and the tx site, we found that RhoA knockdown slowed axon retraction and/or increased early axon regeneration. Moreover, RhoA

knockdown increased the number of axons whose regeneration extended more than 5 mm beyond the injury site at 7 weeks post-tx. Thus, *in vivo* RhoA knockdown may be useful not only to protect spinal-projecting neurons in the brain from retrograde neuronal death, but also to enhance axon regeneration after SCI.

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Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

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Support: NIH R01 NS052741

NMSS RG4958

Mayo Clinic Center for Regenerative Medicine

Title: Targeting protease activated receptor 2 to improve recovery after spinal cord injury

Authors: *M. RADULOVIC[1], H. YOON[2,3], J. WU[2], K. MUSTAFA[1], M. G. FEHLINGS[4], I. SCARISBRICK[1,2,3];
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Abstract: Astrogliosis and inflammation are key integrators of the complex continuum of injury and repair occurring after spinal cord injury (SCI) with molecular drivers representing new therapeutic opportunities. Protease Activated Receptor 2 (PAR2) is a G-protein coupled receptor playing fundamental roles in neural injury, including activities in inflammation and astrogliosis, although its contributions to SCI are essentially unknown. PARs are activated by proteolysis within their extracellular domain revealing a new amino-terminus that binds intramolecularly to elicit intracellular signaling. Thus, PARs enable cells to respond, or to over respond, to rapid changes in the proteolytic microenvironment such as those occurring in traumatic SCI. In this study, we critically evaluated the role and mechanism of action of PAR2 in SCI by determining the impact of PAR2 gene deletion on functional recovery and cellular and molecular signs of pathogenesis in an experimental murine contusion-compression SCI model. Specifically, compression-SCI in PAR2 knockout mice was associated with greater improvements in motor

coordination and strength compared to wild type littermates. Molecular profiling of the injury epicenter, and spinal segments above and below, demonstrated mice lacking PAR2 had significantly attenuated elevations in pro-inflammatory cytokine expression (IL-6, TNF and IL-1β) and in key hallmarks of astrogliosis (GFAP, vimentin, neurocan), but enhanced early elevations in the anti-inflammatory cytokine TGF-β. SCI in PAR2-/- mice was also accompanied by improved preservation of PKC-ϒ-positive corticospinal axons and reductions in GFAP-immunoreactivity, in BIM expression, and in STAT3 signaling. The mechanistic link between PAR2, STAT3 and astrogliosis was investigated in primary astrocytes revealing that the SCI-related serine protease, neurosin (kallikrein 6), promoted IL-6 secretion in a PAR2, MEK1/2- and STAT3-dependent manner. A model is proposed where PAR2-elicited IL-6 secretion drives expression of GFAP, vimentin, and additional IL-6, through canonical STAT3 signaling. Since IL-6 also promoted robust increases in astrocyte PAR2 and neurosin, these data collectively point to an IL-6-driven PAR2 feedback circuit that works hand-in-hand with STAT3 to drive inflammatory-astrogliosis. Given the superior neuromotor recovery observed in PAR2 knockout mice, we suggest that targeting PAR2 to limit inflammation and astrogliosis represents a promising drug target to improve motor outcomes after SCI. Supported by NIH R01 NS052741, NMSS RG4958 and Mayo Clinic Center for Regenerative Medicine.

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Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

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The Miami Project

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Title: A clinically relevant injectable matrix for Schwann cell transplantation following spinal cord injury

Authors: ***S. R. CERQUEIRA**[1], Y.-S. LEE[1], R. CORNELISON[2], C. SCHMIDT[2], M. BUNGE[1];

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Abstract: Schwann cell (SC) transplantation is an extensively studied cell therapy for spinal cord injury (SCI), promoting axon regeneration and myelination. In an attempt to further enhance SC efficacy and maximize tissue repair, ultimately improving functional outcomes, biomaterial-based approaches are being tested in combination with cell therapy. Matrices to support transplanted cell survival, such as Matrigel, create more permissive interfaces that allow enhanced axon extension, but clinically relevant alternatives are needed for translational studies in humans. We chose an injectable biomaterial from acellular peripheral nerve (aPN) to fill the cyst formed after contusion to combine with transplanted SCs. Rat aPNs were obtained using an FDA-approved decellularization method (Hudson *et al., 2004, Tissue Eng 10:1346-58)* and further processed to be injectable and thermally gelling. Injectable aPN yields a solid matrix in physiological conditions with properties similar to native neural tissue. 28 female Fischer rats were subjected to a moderate T8 IH contusion injury (200 kDyn). One week after SCI, $2x10^6$ GFP-labeled SCs were injected into the lesion cavity in either Matrigel or aPN matrix. BBB scoring was performed weekly and grid walk, at 4 and 8 weeks post-transplantation. Longitudinal sections of the spinal cords were immunostained to evaluate SC survival, axon extension, macrophage/microglia activation and the glial scar, and toluidine-blue-stained plastic sections were used for graft integration and axon and myelination assessment. Although no significant differences were observed in BBB scores, BBB subscores revealed a noticeably faster recovery with SC/aPN grafts at 4 weeks, and significantly fewer footslip errors in the grid walk test at 4 weeks. The plastic sections revealed a plethora of SC-myelinated axons within the dense implants in both conditions. Moreover, the SC implants showed no distinct borders with the host tissue. Preliminary immunostaining results indicated no differences in the glial scar at the lesion site, and confirmed the integration of dense implants with the cord. Our results suggest that this clinically relevant injectable aPN matrix is promising to support SC transplantation to promote tissue repair and improve function after SCI in future translational studies. [Funding was supported by NIH NS 09923 (MBB), The Miami Project and the Craig Neilsen Foundation #222456 (CES).]

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Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

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FIFA/F-MARC

ETH funding

Title: Engineered hydrogels supporting fast neurite extension

Authors: *N. BROGUIERE, G. PALAZZOLO, M. ZENOBI-WONG;
ETH Zürich, Zürich, Switzerland

Abstract: Animal derived matrices like fibrin, collagen and matrigel enable encapsulation of neurons with high viability and fast neurite extension. As a result, they have found widespread use as vehicles for the delivery of cells into severed tissues, for gap filling after trauma, and as 3D *in vitro* culture models. Their usefulness is nevertheless limited by their fast degradation, low tunability, poor definition, and/or by the costs and contamination/immunogenicity risks inherent to isolation of proteins from animal tissue. Matrices engineered from polysaccharides or synthetic polymers would be interesting alternatives. We systematically optimized 3 defined gels respectively based on polyethylene glycol (PEG), alginate, and hyaluronan to support similar fast neurite extension in defined, non-immunogenic matrices. Chick dorsal root ganglia (DRGs), rat primary cortical neurons and human induced pluripotent stem cell derived neurons were used as models, covering a wide range of species and neuron types. With all the gel systems and cell types, physical properties were found to be essential to recapitulate high viability and fast neurite extension, whereas specific biological cues such as growth factors and adhesion peptides were unexpectedly found to be unnecessary. In optimized conditions, 2 day viability reached more than 95%, and 3D neurite extension from encapsulated cells occurred at average rates of the order of 100 um/neuron/day. All gels could sustain cultures of cortical neurons for more than a month, yielding synaptically connected and electrically active 3D neural networks. Such neurite permissive hydrogels could find use in the treatment of spinal cord injury, as they are excellent supporting scaffolds for axonal regeneration. They would ideally be used in combination with current strategies to overcome growth inhibition, such as electrochemical stimulation, drug treatment, and stem cell delivery.

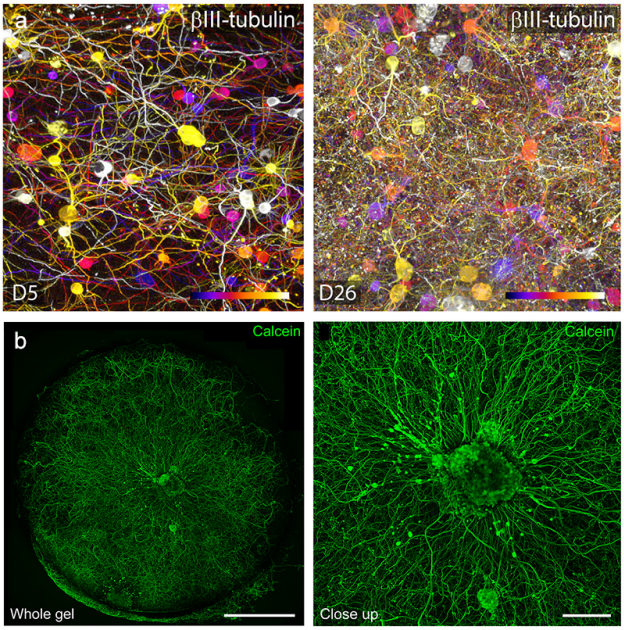

Figure 1. 3D growth in PEG gels (a) Cortical neurons (color scale showing the depth from 0 to 200 um) (b) DRG one month after encapsulation. Scale bars: 50 um (a) 1 mm and 250 um (b)

Disclosures: **N. Broguiere:** None. **G. Palazzolo:** None. **M. Zenobi-Wong:** None.

Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 46.09/D36

Topic: C.10. Trauma

Support: US Department of Defense grant W81XWH-11-1-0668

NIH Grant 5P30 GM103507

Title: Exercise-dependent modulation of neuro-urological health following spinal cord injury

Authors: ***L. R. MONTGOMERY**[1,2], A. N. HERRITY[2,3], S. J. HARKEMA[2,3,4], C. H. HUBSCHER[1,2];
[1]Dept. of Anatom. Sci. and Neurobio., [2]Kentucky Spinal Cord Injury Res. Ctr., [3]Frazier Rehabil. Inst., [4]Dept. of Neurolog. Surgery, Univ. of Louisville, Louisville, KY

Abstract: Spinal cord injury (SCI) affects thousands of people every year leading to dramatic changes in their quality of life. People with SCI must cope with urinary tract dysfunction requiring repeated daily and nightly catheterizations which can lead to further complications such as bladder and kidney infections. Despite bladder dysfunction being a high priority for SCI individuals, the focus of health care professionals is on rehabilitation aimed at optimizing mobility and the remaining musculoskeletal function in these patients. Our lab however, has recently demonstrated that repetitive sensory information generated through task-specific stepping and/or loading can improve non-locomotor functions, including bladder function, following a T9 contusion in male Wistar rats (Ward PJ et al., 2014. J Neurotrauma, 31: 819-833). In order to begin addressing potential underlying mechanisms, the current study was designed to ascertain whether the improvements could be attributed to a global exercise effect on the urogenital system. Male Wistar rats received a T9 contusion injury using the Infinite Horizon (IH) device. Following 2 weeks recovery, animals were randomly assigned into one of 3 groups - quadrupedal locomotion, forelimb exercise, or a non-trained group. All groups of rats were placed in metabolic cages (CLAMS) once a week for 24 hours to monitor water intake and urine output including at baseline (twice pre-injury), post-injury (two weeks), and throughout the 10 week period of daily one hour treadmill training. Following completion of the training period, cystometry data was collected and bladder tissue harvested for qRT-PCR to identify neurotrophin levels (NGF, BDNF, and NT-3). Metabolic cage data indicate that following SCI, mean urine volume increased in all groups of animals, a finding consistent with our previous study on SCI-induced polyuria (Ward P.J. and Hubscher CH, 2012. J Neurotrauma, 29: 2490-2498). However, mid-way through training, the average volume per void for both the quadrupedal and forelimb trained groups significantly decreased, returning towards pre-injury levels. Terminal cystometry data revealed no significant changes between the two exercise groups and sham surgical control animals, although animals in the non-trained group had significantly higher maximum contraction amplitude relative to the sham animals. Neurotrophin levels in the bladder were also altered, with higher levels in the bladders of non-trained animals compared to sham controls. Thus, the positive benefits of exercise on bladder function post-SCI are likely indirect. Currently studies are ongoing in our lab to elucidate the underlying mechanisms.

Disclosures: L.R. Montgomery: None. **A.N. Herrity:** None. **S.J. Harkema:** None. **C.H. Hubscher:** None.

Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 46.10/D37

Topic: C.10. Trauma

Title: Restoring walking ability in individuals with severe spinal cord injury using a closed-loop spinal magnetic stimulation

Authors: *Y. NAKAO[1,2], S. SASADA[3], K. KATO[4], T. MURAYAMA[5], S. KADOWAKI[6], S. YOSHIDA[7], M. IIZUKA[5], T. KOMIYAMA[8], Y. UGAWA[6], Y. NISHIMURA[1,2];
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Abstract: It is a kind of dream for the paraplegic patients to walk again with their legs. The artificial neural connection (ANC) via a closed-loop computer interface is one candidate for accomplishing it because it connects mostly intact spinal cord regions above and below the responsible injury lesion for paraplegia. We have shown that an upper-limb muscle controlled non-invasive magnetic stimulation over the lumbar vertebra was able to induce volitionally-controlled walking behavior in healthy subjects (Sasada et al. 2014). The purpose of present study is to investigate whether or not the ANC is able to restore walking ability and compensate in individuals with severe spinal cord injury (SCI). Five individuals: with severe incomplete (a woman, 19 yo) and complete paraplegia (four men, 21-52 yo), participated in the experiment. The SCI at T3-L1 levels at least half a year before the experiment. All participants were unable to stand and walk independently even after the standard-of-care rehabilitation longer than 3 month. The ANC was accomplished by a computer interface designed to encode the outline of full-wave rectified and moving averaged surface EMG activity from a muscle, and convert it into stimulus pulses. In the present study, the hand-muscle activity was utilized to trigger pulses for TMS pulses which was then delivered over the lumbar vertebra while participants laid in a semi-prone position with their legs suspended. The stimulus intensity was kept constant in each trial, and its range was set at 40-60% of the maximum output of the magnetic stimulator, whereas stimulus frequency was modulated according to the hand-muscle activity between 1 and 20 Hz. All participants could voluntarily initiate and terminate walking-like behavior via the ANC. The induced leg movement enhanced by additional voluntary effort of walking with the ANC. This result implied that even in the individuals who had been clinically diagnosed as complete SCI, the residual corticofugal connection remained. The walking performance was improved by the repeated ANC trials within a day, and ANC trials for several weeks made the performance much better. The ANC successfully compensated for the interrupted descending pathways and can induce walking-like behavior in paralyzed legs in patients with severe SCI. The repeated ANC trials may have driven neural plasticity around the lumbar locomotor circuit that eventually improved walking performance.

Disclosures: **Y. Nakao:** None. **S. Sasada:** None. **K. Kato:** None. **T. Murayama:** None. **S. Kadowaki:** None. **S. Yoshida:** None. **M. Iizuka:** None. **T. Komiyama:** None. **Y. Ugawa:** None. **Y. Nishimura:** 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.; PRESTO.

Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 46.11/D38

Topic: C.10. Trauma

Support: NIH Eunice Kennedy Shriver National Institute Of Child Health & Human Development Grant T32 HD057845

Foundation for Physical Therapy Promotion of Doctoral Studies I Award

Title: The spinal cord lesion: relationship to muscle changes and motor deficits

Authors: *A. C. SMITH[1], T. PARRISH[1], M. HOGGARTH[1], M. WASIELEWSKI[1], H. KIM[2], T. G. HORNBY[2], J. ELLIOTT[1];
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Abstract: Background:Prediction for recovery following incomplete spinal cord injury (iSCI) is a challenging yet important issue for all stakeholders. Magnetic resonance imaging (MRI) is a valuable tool in the prediction of volitional motor return after SCI. Currently, sagittal T2 weighted imaging is commonly used for relating cord lesion characteristics to clinical prognosis. Past literature employed the use of a midsagittal 2-D T2 image, and calculated the length of edema to predict volitional motor function, walking ability, and level of independence following spinal cord injury. Measuring spinal cord lesion volume has recently been explored and related to motor function in an animal model of SCI. However, no study has examined lesion volume in humans. Therefore, the purpose of this work is to quantify and establish relationships between lesion volume, muscle architectural alterations, and volitional motor control following human spinal cord injury. Methods: 5 subjects with chronic cervical iSCI were enrolled in a pilot study (average age = 31 ± 7 years old). Lesion volume was calculated by quantifying the area of edema seen on consecutive sagittal T2 images throughout the cord; then multiplying by slice thickness. Fat infiltration in the lower extremity shank muscles was assessed using a 3 T MRI (Siemens, Erlangen, Germany) with a 2-point Dixon fat/water separation application. Volitional motor

control was quantified using a central activation ratio (CAR), obtained by comparing maximum voluntary isometric plantarflexion torque production with a superimposed peak electrically elicited torque. The data was linearized and Pearson correlation coefficients were calculated to establish relationships between the measures. Results: Linearized lesion volume data was positively correlated with shank muscle fat infiltration (Pearson R = 0.88, P = 0.04) and negatively correlated with plantarflexion CAR values (Pearson R = -0.87, P = 0.05). When correlating edema length versus fat infiltration and CAR values, the relationships were weaker and not statistically significant (Pearson R = 0.57 and -0.56, P = 0.30 and 0.31, respectively). Furthermore, the ability to centrally activate the plantarflexors (CAR) was negatively correlated with amount of fat infiltration in the plantarflexor muscles (Pearson R = -0.93, P = 0.02). Conclusions: Quantifying spinal cord lesion edema volume offers a novel way of investigating spinal cord injury and preliminary results suggest this approach may be superior to measuring edema length, in regards to correlating with peripheral muscle changes and volitional motor deficits. Lesion volume may offer enhanced clinical prognostic value.

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Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 46.12/D39

Topic: C.10. Trauma

Support: Neilson Foundation

Mission Connect

Title: Nociceptive stimulation activates caspase 1 following spinal cord injury by inducing pathologic purinergic signaling

Authors: *J. TURTLE[1], J. A. REYNOLDS[1], M. M. STRAIN[1], Y. J. HUANG[1], S. M. GARRAWAY[2], J. W. GRAU[1];
[1]Texas A&M Univ., Bryan, TX; [2]Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Processes that unfold within the first 48 hours of spinal cord injury (SCI) modulate cell death and determine long-term prognosis. We have shown that nociceptive signals influence these processes and exacerbate secondary damage. Based on studies from a transection model,

we hypothesize that nociceptive input after injury undermines cell survival, impairs recovery of locomotor function, and promotes neuropathic pain. However, the mechanism of impaired recovery following nociceptive input is relatively unknown. Here, we used two different models (uncontrollable electrical stimulation to the tail and peripheral treatment with capsaicin) of nociceptive stimulation (given 24 hours after a moderate spinal contusion at T12) to explore the mechanism of impaired recovery following spinal cord injury. We examined locomotor recovery following injury as well as the underlying cellular mechanisms using immunoblotting of the lesion site. Either peripheral capsaicin injection or uncontrollable tail shock significantly impaired locomotor recovery for at least 28 days when given 24 hours after injury. In addition, nociceptive input increased protein expression of active caspase 1 and increased processing of the pro-inflammatory cytokines IL-1beta and IL-18. These data suggest that after spinal cord injury, nociceptive stimulation promotes the activation of an inflammasome and leads to caspase 1 activation, inflammatory cytokine processing, and potentially pyroptotic cell death. We are exploring the role of purinergic signaling using pharmacologic inhibition of P2X7R with Brilliant Blue G (BBG). If successful, these data would indicate that noxious input soon after spinal cord injury engage a P2X7-dependent purinergic signaling pathway that is capable of activating caspase 1 and promoting cytokine processing. Future work is examining the cell types responsible for inflammasome activation, other mechanisms of cell death, and the effect of drug treatment on functional recovery.

Disclosures: J. Turtle: None. **J.A. Reynolds:** None. **M.M. Strain:** None. **Y.J. Huang:** None. **S.M. Garraway:** None. **J.W. Grau:** None.

Poster

046. Spinal Cord Injury: Restorative Therapeutic Strategies

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 46.13/D40

Topic: C.10. Trauma

Support: NSERC Grant

Title: Impact of sustained sublesional nociception on locomotor recovery following a complete spinal lesion in mice

Authors: *R. JEFFREY-GAUTHIER, K. BERTHELET, M. PICHÉ, H. LEBLOND; Univ. Du Québec À Trois-Rivières, Trois-Rivières, QC, Canada

Abstract: Despite the loss of surpraspinal input following complete spinal cord section, central pattern generators (CPG) located in the lumbosacral spinal cord can generate treadmill locomotion, in various animal models. By enhancing cutaneous and proprioceptive inputs to CPG, treadmill training promotes locomotor recovery. Yet, it is not known if nociceptive inputs may alter this locomotor recovery. In spinal rats, nociceptive stimuli disrupt spinal regulation of the flexion reflex during conditioning. However, the impact of nociception on more complex tasks, including locomotion, is not known. The aim of this study was to investigate the impact of sustained sublesional nociception on locomotor recovery following a complete spinal section at T8 in mice. Sustained sublesional nociception in the form of chronic inflammation was induced immediately following complete spinal section by injecting complete Freund adjuvant (CFA) in paraspinal muscles bilaterally, in an experimental group of mice (CFA, n=7), while animals in the control group (CTL, n=8) received no injection. Animals of both groups were then trained daily on a treadmill until locomotor recovery was achieved. Interlimb coordination and detailed kinematic analysis of left hindlimb were assessed by monitoring the step cycle pattern and articular excursions using high speed camera recordings of treadmill locomotion at day 2, 8, 15, 21 and 28 post-lesion. The kinematic analysis showed that CFA mice exhibited a lack of articular excursion throughout the step cycle, which resolved by day 28. This deficit was associated with a large step length decrease observed until day 15. This altered kinematics was observed minimally in CTL mice, but was still sufficient to induce a hindpaw drag during the swing phase. However, paw drag vanished in the late swing phase by day 15 for CTL mice while it was observable until day 28 for CFA mice. Moreover, interlimb coordination measured with the left-right step cycle coupling was more affected in CFA mice. Normally patterned locomotion was not observable before day 28 in this group, while CTL mice exhibited normal left-right coupling by day 15. Altogether, these findings suggest that sustained sublesional nociceptive activity significantly alters locomotor recovery following complete spinal lesion in mice. Since sustained nociception presumably occurs in human patients with spinal cord injury, these results bring new insights for human research aiming at optimizing locomotor recovery.

Disclosures: **R. Jeffrey-Gauthier:** None. **K. Berthelet:** None. **M. Piché:** None. **H. Leblond:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.01/D41

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

Support: NIH Grant DA013137

NIH Grant DA031604

NIH Grant HD043680

NIH Grant GM087140

Title: HIV-1 transgenic rat: alterations in naturally rewarding voluntary wheel running and medium spiny neurons of the nucleus accumbens

Authors: *M. N. CRANSTON, R. M. BOOZE, S. B. HARROD, R. F. ROSCOE, Jr., C. F. MACTUTUS;
Psychology, Univ. of South Carolina, Columbia, SC

Abstract: Motivational alterations in HIV-1+ individuals are associated with decreased performance on tasks involving frontal-subcortical circuitry and the nucleus accumbens. In the present study, the HIV-1 transgenic (Tg) rat was used to assess long-term HIV-1 viral protein exposure on motivated behavior using voluntary wheel running and on medium spiny neurons (MSNs) in the nucleus accumbens. Adult ovariectomized female HIV-1 Tg animals (n=21) to F344 controls (n=26) were pair-housed under a 12:12 light/dark cycle. Voluntary running was measured with 34cm-diameter running wheels for 60 minutes per day for 3 months. There were no significant differences between HIV-1 Tg and F344 control rats in voluntary wheel running during the light phase. Animals were then run in the nocturnal phase of their light/dark cycle. We found that the HIV-1 Tg animals initially ran more than F344 controls and reached an earlier asymptotic plateau; however, F344 controls continued to escalate their overall running and surpassed the stabilized HIV-1Tg group after ~4 weeks of nocturnal running, until reaching their asymptotic plateau at week 11. Maximal running speed was not different between the HIV-1 Tg and F344 controls. MSNs in the nucleus accumbens were ballistically-labeled with the indocarbocyanine dye DiI, followed by 3-dimensional dendritic spine analysis. Decreased length and volume of MSN dendritic spines in HIV-1 Tg rats, relative to F344 controls, suggests a reduction of longer spines and an increase in shorter, less projected spines. Collectively, selective alterations in the rewarding efficacy of voluntary wheel running and altered dendritic spines of the MSNs, indicates a dysfunction of the motivational circuitry within the HIV-1 Tg rat brain. These findings may help elucidate mechanisms of motivational alterations in HIV-1+ individuals.

Disclosures: M.N. Cranston: None. **R.M. Booze:** None. **S.B. Harrod:** None. **R.F. Roscoe:** None. **C.F. Mactutus:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.02/D42

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

Support: NIH Grant T32 DA007027

NIH Grant R01 DA018633

Title: nNOS positive interneuron subpopulations in CA1 subregions are selectively vulnerable to HIV-1 Tat

Authors: W. D. MARKS[1], C. J. SCHIER[1], *K. F. HAUSER[1], S. FITTING[2];
[1]Pharmacol. & Toxicology, Virginia Commonwealth Univ., Richmond, VA; [2]Psychology Dept., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: The hippocampus is known to be significantly disrupted in neuro-acquired immunodeficiency syndrome (neuroAIDS). This region is comprised of a large variety of interneuron subtypes with varying structures and functions that give rise to a complex processing network. Moreover, hippocampal pathology has been suggested to underlie certain HIV-associated neurocognitive deficits and HIV-1 Tat is thought to contribute markedly to the pathological processes observed in neuroAIDS. We hypothesize the diverse interneuron subtypes are selectively vulnerable to HIV-1 Tat and that this selective susceptibility will underlie deficits in network function. To test this hypothesis, neuronal vulnerability was assessed in GFAP-driven, doxycycline-inducible Tat transgenic mice, with control mice lacking only the tat transgene. Subregions of the hippocampus CA1 were probed in brain slices using antibodies for neuronal markers, including parvalbumin (PV), neuronal nitric oxide synthase (nNOS), neuropeptide Y (NPY), and neuronal nuclear marker (NeuN), combined with fluorescent tagged secondary antibodies. No significant effect was found for Parvalbumin positive cells in the CA1. There were regional and subtype difference observed in nNOS positive neurons. The percentage of nNOS-immunoreactive interneurons without NPY immunoreactivity was significantly decreased in the pyramidal layer of the CA1 region of the hippocampus, as was the percentage of nNOS-immunoreactive interneurons in the stratum radiatum of CA1 in Tat expressing animals compared to controls. The results indicate that at least one subset of CA1 nNOS-expressing interneurons is selectively vulnerable to HIV-1. This finding reveals novel structural deficits in hippocampal circuitry that are likely to underlie behavioral deficits in neuroAIDS.

Disclosures: W.D. Marks: None. **C.J. Schier:** None. **K.F. Hauser:** None. **S. Fitting:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

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

Support: NIH Grant MH085607

State of Florida

Title: Exposure to HIV-1 Tat protein modulates forebrain glutamate levels and increases depression-like behavior

Authors: *J. P. MCLAUGHLIN[1,2], M. L. GANNO[2], S. O. EANS[1,2], J. M. MEDINA[1,2], H. M. STACY[2], T. E. GILLIS[3], A. N. ROCK[3], D. MINTZOPOULOS[3], J. J. PARIS[4,2], M. J. KAUFMAN[3];
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Abstract: Aims: While exposure to the HIV-1-accessory protein Tat increases striatal dopamine levels and anxiety-like behaviors, the functional consequences of Tat protein on forebrain neurotransmitter levels and behavioral depression are little known. Accordingly, we hypothesized that HIV-1 Tat expression in brain would modulate glutamate levels and promote behavioral depression. Methods: In the GT-tg bigenic mouse model, which enables controlled brain-selective Tat expression by activation of a doxycycline (Dox) promotor, we used magnetic resonance spectroscopy (MRS) to determine whether Tat protein alters medial frontal cortex (MFC) glutamate and N-acetylaspartate (NAA) levels. Furthermore, we tested the effects of Tat protein on depression-like behavior in the tail-suspension test (TST) and saccharin consumption. Additional mice treated daily with the NMDA-receptor antagonist ifenprodil during the induction of Tat protein were also examined for depression-like behavior. Results: Western blot analysis confirmed that the expression of Tat protein in GT-tg bigenic mice correlated with dose and duration of Dox treatment, lasting between 7 and 14 days. MRS scans detected trending and significant MFC decreases in glutamate and NAA, respectively, in GT-tg mice treated 7d with Dox versus the baseline measures in these mice. In initial behavioral testing, GT-tg bigenic mice expressing Tat protein for 7 d demonstrated significant increases in time spent immobile during the TST as well as a significant decrease in saccharin consumption as compared to saline-treated littermates lacking Tat, both characteristic of depression-like behavior. Time spent immobile in the TST was dependent on the magnitude of exposure to Tat protein, as demonstrated by the dose and duration of Dox treatment to induce Tat-protein, as was the persistence of the effect. Of

interest, daily treatment with ifenprodil (10 mg/kg/d, i.p.) reduced the time Tat-induced GT-tg mice spent immobile in the TST for the first 7 days after Tat induction. Conclusions: Overall, these data suggest that expression of HIV-1 Tat protein in the mouse brain is sufficient to increase behavioral depression. Moreover, the Tat-induced decrease in MRS metabolites suggests biological mechanisms by which HIV infection may increase the vulnerability to depression, which could be exploited via specific therapeutic interventions such as glutamate-receptor antagonists.

Disclosures: **J.P. McLaughlin:** None. **M.L. Ganno:** None. **S.O. Eans:** None. **J.M. Medina:** None. **H.M. Stacy:** None. **T.E. Gillis:** None. **A.N. Rock:** None. **D. Mintzopoulos:** None. **J.J. Paris:** None. **M.J. Kaufman:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.04/D44

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

Support: F32 NS060657

Title: A unique subset of CD8 T cells (CD4DIMCD8Bright T Cells) is associated with HIV control in the CNS and better neuropsychological function

Authors: ***M. H. RICHARDS**[1], V. LUTGEN[2], S. NARASIPURA[2], L. AL-HARTHI[2]; [1]Immunol. and Microbiology, Rush Univ. Med. Ctr., Hanover Park, IL; [2]Immunol. and Microbiology, Rush Univ. Med. Ctr., Chicago, IL

Abstract: HIV invades the brain within two weeks of infection and can lead to a spectrum of neurocognitive dysfunction termed HIV-Associated Neurocognitive Disorders (HAND). We previously determined that a subset of CD8+ T cells, $CD4^{dim}CD8^{bright}$ T cells (referred to as Double Positive (DP) T cells), is enriched in anti-HIV responses. We evaluated the role of DP vs. single positive (SP) CD8+ T cells in CNS HIV control using HIV+ NOD/SCID/IL-2rcγ-/- (NSG) mice reconstituted with human PBMCs (NSG-huPBMCs) and a cohort of HIV- infected patients on and off antiretroviral therapy. Both DP and SP CD8+ T cells are found in the brain of NSG-huPBMCs within three weeks of infection. Given that DP T cells constitute 3-5% of CD8+ T cells in healthy individuals, these cells could have migrated to the brain as DP T cells or the brain environment may facilitate CD4 induction on CD8+ T cells. To assess whether the brain environment could induce the DP T cell phenotype, CD8+ SP T cells were intracranially injected

into the brain of NSG mice. We report that CD4 expression was induced by 10-fold, indicating that the brain microenvironment can induce the DP T cell phenotype. Further, percentage of DP T cells was inversely correlated with HIV-gag mRNA (r_s=-0.61, p≤0.001) in the mouse brain while no correlation was established between CD8SP and HIV-gag mRNA (R=-0.23,p≤0.3). We extended these studies to the human condition by evaluating the relationship between CSF DP and CD8SP T cells and CSF viral load (VL) among 40 HIV- infected patients who were either on therapy and controlling HIV (HIV RNA < 500 copies/mL; n=11), on therapy but failing to control HIV (>500 copies/mL; n= 12), or not on therapy (n=17). CSF DP T cells inversely correlated with CSF VL (r_s = -0.53565, p≤0.0019) across all groups of HIV-infected patients. Conversely, CSF CD8SP T cells positively correlated with CSF VL (r_s = +0.55913, p≤0.0011) across all groups of HIV infected patients. Further, we evaluated the relationship between DP and CD8SP T cells and Neurospychological Z -4 score (NPZ-4). NPZ is a composite Z score for a short battery of tests (grooved pegboard, timed gait, finger tapper, and WAIS-R digit symbol) which evaluate memory, psychomotor speed, and executive function. We show that percentage of CSF DP T cells is positively correlated with neuropsychological function (r_s=+0.45341, p≤ 0.008) and no such association exists for CD8SP T cells (r_s=-0.18642, p≤ 0.2911). Collectively, these data indicate that a higher frequency of DP T cells and not CD8SP T cells is associated with HIV control in the CNS and better neurocognitive function.

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Poster

047. HIV Neuroinflammation

Location: Hall A

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Program#/Poster#: 47.05/D45

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

Support: National Research Foundation of South Africa

Title: Effect of antiretroviral therapy on HIV-1 tat protein-induced neurotoxicity

Authors: *W. M. DANIELS, S. ZULU, V. RUSSELL, M. MABANDLA;
Univ. of Kwazulu-Natal, Durban, South Africa

Abstract: Previous studies have demonstrated that the viral protein Tat is neurotoxic. Its damaging effects are mediated through increasing reactive oxygen species (ROS) and the excessive production of pro-inflammatory cytokines. The objective of this study was to

investigate whether a combination of antiretroviral drugs is effective in reducing the toxic effects of Tat. Male Sprague-Dawley rats were divided into four groups (n=10). Each rat received bilateral intrahippocampal injections of either Tat (5µg/10µl) or vehicle, followed 7 days later by a combination of antiretroviral drugs (Zidovudine 12mg/kg, Lamivudine 6mg/kg and Efavirenz 24mg/kg) or saline injected intraperitoneally, twice a day, for 7 days. After treatment, animals were sacrificed and hippocampal tissue was collected for analysis of active caspase-3,4-hydroxynonenal (NHE), tumor necrosis factor alpha (TNF-α), phosphorylated extracellular signal-regulated kinase (pERK) and synaptophysin. Tat increased caspase-3 levels in the hippocampus that was decreased by antiretroviral treatment. Tat increased HNE, a marker of lipid peroxidation and reduced hippocampal synaptophysin. The latter Tat-induced effects were not reversed by antiretroviral treatment. The antiretroviral treatment activated the pERK pathway and increased TNF-α levels in hippocampal tissue, independent of Tat infusion. Our findings showed that antiretroviral drugs reversed Tat-induced activation of caspase-3, reducing apoptosis but did not reverse Tat-induced increase in lipid peroxidation and the synaptic marker, synaptophysin. The evidence suggests that the combination of antiretroviral drugs may be toxic, elevating hippocampal pERK and TNF-α levels. However, these effects could also be beneficial to the individual, since TNF-α has been shown to inhibit viral replication. The present results provide novel insight into the mechanism of antiretroviral action.

Disclosures: **W.M. Daniels:** None. **S. Zulu:** None. **V. Russell:** None. **M. Mabandla:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.06/D46

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

Support: NIH grant R01 NS077873

Title: Involvement of potassium channel KV1.3 in HIV Tat-induced oligodendrocyte/myelin injury

Authors: ***H. LIU**, J. LIU, G. TU, H. XIONG;
Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Brain white matter (WM) is composed of bundles of neuronal axons myelinated with oligodendrocytes (Ols), the myelin-forming cells. Without infection of neuronal cell body and axons, HIV-1 does cause myelin/WM injury and myelin pallor is one of well-established

pathological features in HIV-1-infected brain. Myelin/WM damage has indeed been found not only in patients with HIV-associated cognitive disorders (HAND), but also in HIV-1 positive asymptomatic individuals. How HIV-1 induces myelin/WM damage and consequent cognitive impairment remain incomplete understood. It has been shown that Ols are sensitive to viral proteins such as HIV Tat (a key viral transactivator of transcription) and that a decrease in number of Ols is one of the manifestations of myelin damage. It has also been shown activation of voltage-gated K^+ (K_V) channels mediates apoptosis in various types of cells. We hypothesize that HIV Tat induces Ol injury via activation of $K_V1.3$ channel because $K_V1.3$ is the most predominant K_V channel expressed in Ols and potentially involved in regulation of Ol development. To test this hypothesis, we studied the effects of Tat on whole-cell outward K^+ current conducted by $K_V1.3$ in Ol lineage cells and examined association of Tat alteration of $K_V1.3$ current and Tat-mediated Ol injury. Our results showed incubation of Ols with Tat enhanced outward $K_V1.3$ current and the levels of $K_V1.3$ expression, but decreased the expression of myelin basic protein. Tunel staining revealed that Tat caused Ol apoptosis *in vitro* and decreased axonal myelination *ex vivo.* The Tat-associated alterations were blocked either by specific $K_V1.3$ blockers 5-(4-phenoxybutoxy) psoralen, Margatoxin or by siRNA knockdown of $K_V1.3$ gene. Taken together, our results suggest that Tat-induced Ol/myelin damage via $K_V1.3$ and blockade of $K_V1.3$ may have a therapeutic potential.

Disclosures: **H. Liu:** None. **J. Liu:** None. **G. Tu:** None. **H. Xiong:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.07/D47

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

Support: NIH grant R01NS077873

Title: Methamphetamine potentiates HIV-1gp120-induced microglia neurotoxicity via potassium channel $K_V1.3$

Authors: *J. LIU, H. LIU, E. XU, G. TU, H. XIONG;
Dept. of Pharmacol. and Exptl. Neurosci., Univ. NE Med. Ctr., Omaha, NE

Abstract: Human immunodeficiency virus (HIV) brain infection causes microglia (MG) activation and release of pro-inflammatory molecules leading to the development of HIV-associated neurocognitive disorders (HAND) and methamphetamine (Meth) abuse exacerbates

HAND. However, the mechanisms underlying Meth exacerbation of HAND are not fully understood. Voltage-dependent potassium (K_V) channels have recently gained attention in the regulation of MG functionality and MG express several types of K_V channels such as outward delayed rectifiers $K_V1.5$ and $K_V1.3$. We hypothesize that Meth potentiates HIV-1-induced MG neurotoxic activity via activation of MG $K_V1.3$. To test this hypotheses, we studied co-morbid effects of Meth and HIV-1 glycoprotein 120 (gp120) on MG $K_V1.3$ expression, $K_V1.3$ current and involvement of $K_V1.3$ in MG production of neurotoxins. Our results revealed that Meth potentiated gp120 enhancement of $K_V1.3$ protein expression, $K_V1.3$ current and MG production of neurotoxins leading to neuronal apoptosis in a does dependent matter, which were blocked by pretreatment of MG with specific $K_V1.3$ channel blocker 5-(4-Phenoxybutoxy)psoralen (PAP), or by broad spectrum K_V channel blocker, 4-AP, indicating an involvement of $K_V1.3$ in Meth/gp120-induced MG neurotoxic activity. Further studies were carried out to identify intracellular signaling for Meth/gp120/$K_V1.3$-associated MG neurotoxic activity. Our data showed that Meth and gp120 activated caspase-8 and caspase-3/7 sequentially and increased their activity. Blockage of caspase-8 and caspase-3/7 by their specific inhibitors significantly decreased MG secretion of pro-inflammatory cytokines and resultant neurotoxic activity. Interestingly, blockage of $K_V1.3$ by specific blockers largely mitigated Meth/gp120 enhancement of caspase-8 and caspase-3 activity, resulting in an attenuation of Meth/gp120-induced MG neurotoxicity. Taken together, our results demonstrated an involvement of MG $K_V1.3$ in the mediation of a co-morbid effect of Meth/gp120 on MG neurotoxic activity. Our results also suggest that blockade of MG $K_V1.3$ could be a potential therapeutic strategy in the suppression of Meth/HIV-associated MG neurotoxicity.

Disclosures: **J. Liu:** None. **H. Liu:** None. **E. Xu:** None. **G. Tu:** None. **H. Xiong:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

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Program#/Poster#: 47.08/D48

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

Support: PO1 MH64570

T32NS007489

Title: Lrrk2 kinase inhibition attenuates inflammasome priming in microglia

Authors: *P. MILLER-RHODES, C. KIM, S.-M. LU, H. GELBARD;
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Abstract: Human immunodeficiency virus (HIV) associated neurocognitive disorder (HAND) encompasses a wide variety of neurologic disease observed in up to half of HIV-positive patients. HAND varies in its severity and persists despite combination antiretroviral therapy, regardless of its ability to penetrate the central nervous system. Despite its prevalence, the neuropathophysiology of HAND is not well understood, and adjunctive therapies remain an unmet medical need. A literature based on neuropathologic studies and experimental laboratory models identifies microglial activation and pro-inflammatory cytokine secretion as hallmarks of HAND; and further suggests a role for inflammasomes in this disorder. Inflammasomes are multiprotein complexes that mediate the processing and release of the proinflammatory cytokines IL-1β and IL-18, which are secreted signaling molecules involved in the inflammatory response, via the activation of caspase-1. Recent research indicates that HIV-1 infection of monocytes initiates inflammasome "priming" by upregulating the expression of pro-IL-1β and pro-IL-18. Current work in our lab has focused on characterizing the therapeutic effects of inhibition of leucine-rich repeat kinase type 2 (LRRK2) in our *in vitro* and *in vivo* HAND models. Recently, we identified a role for LRRK2 in modulating inflammasome priming in a murine microglial cell line (BV-2), in which we used qRT-PCR to measure the time course of pro-IL-1β expression following lipopolysaccharide (LPS) treatment of BV-2 cells. After identifying peak pro-IL-1β expression six hours after LPS exposure, we then tested whether pharmacological inhibition of LRRK2 could attenuate this process. We found that LRRK2 kinase inhibition results in a robust inhibition of pro-IL-1β expression following treatment with inflammatory stimuli relevant to HIV-1 infection. These results suggest a novel pathway through which inhibition of LRRK2 exerts therapeutic effects in the context of HAND.

Disclosures: P. Miller-Rhodes: None. **C. Kim:** None. **S. Lu:** None. **H. Gelbard:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.09/E1

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

Support: DA013137

DA031604

HD043680

NIDA/IAS

Title: Cocaine- and methamphetamine-induced disruption of striatal dopamine and medium spiny neurons in HIV-1 transgenic rats

Authors: *M. JAVADI PAYDAR, R. F. ROSCOE, Jr, C. F. MACTUTUS, R. M. BOOZE; Univ. of South Carolina, Columbia, SC

Abstract: HIV-associated neurocognitive disorders (HAND) are associated with serious complications, and addictive drugs such as cocaine (COC) and methamphetamine (METH) exacerbate these clinical manifestations. In the present experiments, we investigated the consequences of COC- and METH-induced DA release and transport in striatal brain slices from HIV-1 transgenic (Tg) and F344 control female rats during diestrous using fast-scan cyclic voltammetry. We examined DA reuptake and release in control, HIV-1, COC-treated (5μM), HIV-1 + COC-treated, METH-treated (8 μM) and HIV-1 + METH-treated striatal slices. In striatal slices from F344 control animals, COC and METH treatment produced a significant increase in DA reuptake time (T80), relative to untreated control slices. In HIV-1 Tg striatal slices, DA reuptake was significantly prolonged following METH; however, DA reuptake time remained unchanged after treatment with COC. DA levels were altered in HIV-1 Tg striatal slices, as DA in HIV-1 Tg slices was significantly increased relative to the F344 control striatum. Although DA was not affected in METH-treated controls, DA significantly increased in COC-treated controls. In HIV-1 Tg, DA decreased two-fold following exposure to both COC and METH compared to untreated HIV-1 Tg slices. Analysis of DiOlistically-labeled medium spiny neuron (MSN) dendritic spines from HIV-1 Tg brain slices treated with cocaine had the longest dendritic spines with the widest head diameters, relative to F344 controls. Taken together, the current study provides evidence for the dysfunctional role of DAT in mediating DA reuptake within the striatum of HIV-1 Tg rats following exposure to COC and METH. Collectively, dysfunction of the DAT protein and altered dendritic spine morphology of the MSNs, suggests a dynamic, functional disruption of the cocaine reward circuitry within the HIV-1 Tg rat brain.

Disclosures: M. Javadi Paydar: None. **R.F. Roscoe:** None. **C.F. Mactutus:** None. **R.M. Booze:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.10/E2

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

Support: R01 NS060632

R01 DA 033966

K24 MH097673

P50 DA26306

Title: Dickkopf-related protein 1 is associated with hiv-associated neurocognitive impairment

Authors: *C. YU[1], M. SEATON[1], S. LETENDRE[2], R. HEATON[2], L. AL-HARTHI[1];
[1]Dept. of Immunol. and Microbiology, Rush Univ., Chicago, IL; [2]UCSD, San Diego, CA

Abstract: Dickkopf-related protein 1 (DKK1) is a soluble antagonist of the Wnt/b-catenin pathway. We demonstrated that b-catenin is a critical regulator of the glutamate/glutamine cycle in astrocytes, diminished Wnt/b-catenin signaling perturbs their neuroprotective properties, and methamphetamine (Meth, a frequent co-morbid of HIV infection) inhibits b-catenin signaling. We hypothesized that increased DKK1 would diminish b-catenin signaling and increase the risk for neurocognitive impairment (NCI) in HIV+ subjects. To assess the relationship between plasma DKK1 and NCI, plasma samples from 41 HIV+ and 43 HIV- adults were obtained from the UCSD TMARC cohort. DKK1 and MCP-1 plasma levels were measured by immunoassay. A neuroinflammatory chemokine, MCP-1 was included as a comparison marker. Meth usages were self-reported. All subjects were assessed using a standardized comprehensive NC battery that adhered to Frascati guidelines. NC performance was summarized using the global deficit score method. Levels of MCP-1 ($p = 0.02$) but not DKK1 ($p = 0.65$) were higher in HIV+ subjects than in HIV- subjects. Higher DKK1 levels ($p = 0.02$) were associated with meth density/use frequency in all subjects. Among HIV+ subjects, higher levels of DKK1 ($d=0.63$, $p = 0.05$) but not MCP-1 ($p = 0.59$) were associated with NCI, those who had plasma DKK1 levels of at least 1,129 pg/ml had a 6.0-fold increased odds of having NCI (75% vs 33%, 2-tail FET $p=0.04$). The effect size was large and the association between DKK1 and NCI was highly specific (92%). In comparison, recursive partitioning failed to identify a statistically significant threshold value for MCP-1. Multivariable analysis among HIV+ subjects identified that the association between higher DKK1 levels and NCI remained statistically significant after accounting for the effects of nadir CD4+ T-cell counts, drugs of abuse, and ART use. These findings underscore the potential specificity of DKK1 as a biomarker for NCI in HIV+ adults.

Disclosures: C. Yu: None. **M. Seaton:** None. **S. Letendre:** None. **R. Heaton:** None. **L. Al-Harthi:** None.

Poster

047. HIV Neuroinflammation

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.11/E3

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

Support: NS 086426

MH 101019

NS 083164

Title: HIV induces a unique neurotoxic phenotype in human monocyte-derived macrophages that is suppressed by nerve growth factor and the neurotrophin ligand, LM11A-31

Authors: *K. S. WILLIAMS[1], J. A. SEAWELL[2], V. ZHURAVLEVA[3], R. B. MEEKER[3]; [1]Neurol., Univ. of North Carolina Sch. of Med., Chapel Hill, NC; [2]Neurol., [3]UNC Chapel Hill, Chapel Hill, NC

Abstract: When macrophages or microglia encounter HIV they secrete neurotoxic substances which cause neural damage. The damage includes a destabilization of intracellular calcium, appearance of varicosities in the dendrites, actin aggregation and dendritic pruning and mimics the pathology seen in other neurodegenerative diseases such as Alzheimer disease. One approach to prevent neurotoxicity is to reduce the activation of the macrophages by HIV. However, the processes that lead to toxin secretion and the nature of the toxins are not well understood. Neurotrophin receptors on macrophages may play a role in the control of toxic activity by altering the signaling machinery essential for HIV-induced activation. In particular, differential signaling by pro-neurotrophins versus mature neurotrophins in macrophages may contribute to neuronal damage in a fashion that parallels the proposed actions of these neurotrophins in neurons. NGF and a potential therapeutic small non-peptide molecule that resembles loop 1 of NGF, LM11A-31, suppressed neurotoxin secretion during HIV stimulation of human monocyte derived macrophages (hMDM) whereas proNGF facilitated neurotoxin production as assessed by calcium destabilization in primary rat cortical neurons. Specific actin rich structures in hMDM correlated with neurotoxin production. Cultures of hMDM possessing podosomes were more neurotoxic than cultures containing ruffled hMDM. Podosome formation in macrophages was induced by HIV and suppressed by treatment with NGF or LM11A-31. The latter stimuli typically resulted in macrophages with membrane ruffling. Blocking the p75NTR but not TrkA, during HIV stimulation prevented HIV induced podosome formation. These morphological features correlated closely with high (ruffles) or low (podosomes) levels of intrinsic calcium spiking in hMDM. HIV suppressed calcium spiking which was restored in the presence of NGF or LM11A-31. Calcium spiking was reduced by proNGF. A parallel assessment of the conditioned medium showed a subset of secreted proteins that were increased by HIV and that

correlated with calcium destabilization in neurons. NGF and LM11A-31 co-stimulation with HIV reversed the secretion of these proteins while proNGF often increased their secretion and induced a distinctive phenotype. These data suggest that NGF and LM11A-31 have the ability to control the neurotoxic activity induced by HIV while proNGF may enhance neuorinflammation. Targeting neurotrophin receptors on macrophages with LM11A-31 may be a novel therapeutic avenue to suppress neuroinflammation during HIV associated cognitive disorders and other neuroinflammatory diseases.

Disclosures: K.S. Williams: None. **J.A. Seawell:** None. **V. Zhuravleva:** None. **R.B. Meeker:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

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Program#/Poster#: 47.12/E4

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

Support: a National Institute on Drug Abuse (NIDA) Center award to the Translational Methamphetamine AIDS Research Center (TMARC, P50 DA26306

Training in Research on Addictions in Interdisciplinary NeuroAIDS NIDA Grant (TRAIN, T32 DA031098

Interdisciplinary Research Fellowship in NeuroAIDS

Title: Independent and combined effects of methamphetamine and HIV gp120 protein on neural microstructure in mice using diffusion tensor imaging

Authors: B. S. MCKENNA[1], *G. G. BROWN[4,2], S. ARCHIBALD[2], M. SCADENG[3], R. BUSSELL[3], J. KESBY[2], A. MARKOU[2], S. SEMENOVA[2];
[1]Dept. of Psychiatry, [2]Psychiatry, [3]Radiology, Univ. of California San Diego, La Jolla, CA; [4]VASDHS, San Diego, CA

Abstract: Methamphetamine (METH) abuse is common among individuals infected by human immunodeficiency virus (HIV). METH and HIV cause injury to both cortical and subcortical brain regions. However, it is unclear what the independent versus combined effects of HIV and METH are on brain microstructure. The expression of HIV gp120 protein induces neuropathology in mice similar to HIV-induced pathology in humans. We examined the independent and combined effects of HIV gp120 protein and METH on the brain using *in vivo*

diffusion tensor imaging (DTI) with 27 gp120-transgenic and 27 non-transgenic mice, of which 14 in each group underwent an escalating METH binge or saline control regimen. Mean diffusivity (MD) served as a measure of microstructural integrity of neural tissue. DTI was conducted 3-4 months post-regimen when mice were 9-10 months old. MD maps were calculated and data were aligned to atlas space for analyses. Multiple linear regression with an orthogonal set of contrast codes designed to examine independent and combined effects were used with the following comparisons: 1) control mice vs. the three experimental groups; 2) dual gp120/METH vs. gp120 only and METH only mice; and 3) gp120 only vs. METH only mice. A corrected p-value for multiple comparisons of 0.05 was used for all analyses. Compared to the control group, the combined METH, gp120, and dual gp120/METH groups demonstrated increased MD within bilateral hippocampi, genu of the corpus callosum, and posterior isocortex. Within hippocampi similar increases in MD were found in each experimental group. Compared to METH only and gp120 only groups, dual gp120/METH mice had increased diffusivity in bilateral midbrain, right hypothalamus, entorhinal cortex, globus pallidus, and caudate. Increased MD was observed in METH only relative to gp120 only within bilateral caudate, right internal capsule, left globus pallidus and hippocampus; whereas increased diffusivity in gp120 only relative to METH only was observed in right midbrain, left thalamus, and genu of the corpus callosum. The results highlight both spatially distinct independent and combined effects of METH and HIV protein gp120 on DTI-measured MD. Prior METH exposure had a larger impact on the striatum relative to gp120, whereas gp120 had larger relative impact on midbrain and diencephalic nuclei. Combined METH and gp120 led to further increases in MD in some, but not all these regions. Hippocampi were affected by both METH and gp120, but not additively. Increased mean diffusion in these regions may reflect damage to cell bodies and/or their processes suggesting METH and gp120 may impact the brain through overlapping and distinct mechanisms.

Disclosures: **B.S. McKenna:** None. **G.G. Brown:** None. **S. Archibald:** None. **M. Scadeng:** None. **R. Bussell:** None. **J. Kesby:** None. **A. Markou:** 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.; I have received contract support from Forest Laboratories and Astra-Zeneca during the last 3 years.. **S. Semenova:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

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Program#/Poster#: 47.13/E5

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

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NIH Grant T32-MH078795

Title: The critical role of the inflammatory cytokine interleukin-1 beta and the iron storage protein ferritin heavy chain in synaptodendritic injury during HIV infection

Authors: *L. FESTA[1], C. GUTOSKEY[2], A. GRAZIANO[1], B. WATERHOUSE[2], O. MEUCCI[1];
[1]Pharmacol. & Physiol., [2]Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Despite the introduction of combination antiretroviral therapy (cART), HIV-associated neurocognitive disorders (HAND) continue to persist. While the molecular mechanisms of HAND are not fully understood, both viral (e.g. HIV gp120) and host (e.g. inflammatory cytokines) factors can contribute to neuronal dysfunction, including synaptodendritic injury. Here, we propose that HIV proteins and inflammatory cytokines induce synaptic damage by upregulating neuronal expression of the iron storage protein ferritin heavy chain (FHC), a novel negative regulator of the homeostatic chemokine/receptor pair CXCL12/CXCR4. We previously found that the HIV envelope protein, gp120, can regulate neuronal levels of FHC, which is mediated by gp120-evoked release of the inflammatory cytokine, IL-1 beta, from glial cells. In order to further investigate the consequences of FHC alterations, we utilized two noninfectious rodent models of HAND (HIV-Tg and gp120-treated rats). We observed significant reductions in both dendritic spine density and basal dendrite branching in layer II/III pyramidal neurons of the medial prefrontal cortex (mPFC), suggesting decreases in neuronal complexity and connectivity. In line with this, gp120-treated rats exhibited deficits in reversal learning, a measure of cognitive flexibility, and this was negatively correlated with dendritic spine density (Pearson r= -0.8036). Interestingly, elevated levels of FHC were observed in frontal cortex lysates in the same HIV-Tg rats used for spine analysis. Furthermore, in neuronal/glial bilaminar co-cultures, the relative FHC staining intensity in neurons was negatively associated with dendritic spine density (Pearson r= -0.7441), regardless of treatment group (vehicle or gp120). Importantly, gp120 was unable to decrease dendritic spine density in FHC-deficient neurons, thus establishing FHC's critical role in the spine changes mediated by the envelope protein. Taken together, with our previous findings in HIV patients and SIV-infected primates, these data validate FHC's critical role in synaptodendritic injury and point to IL-1 beta as a valid therapeutic target.

Disclosures: **L. Festa:** None. **C. Gutoskey:** None. **A. Graziano:** None. **B. Waterhouse:** None. **O. Meucci:** None.

Poster

047. HIV Neuroinflammation

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

Support: NIH Grant NS086426

NIH Grant NS083164

UNC Summer Undergraduate Research Program

Title: Neurotrophin/CXCR4 receptor interactions regulate calcium activity and HIV-induced neurotoxin secretion in human monocyte-derived macrophages

Authors: V. ZHURAVLEVA, K. WILLIAMS, J. SEAWELL, *R. B. MEEKER;
Neurol., Univ. of North Carolina, Chapel Hill, NC

Abstract: Therapeutic approaches designed to restore cognitive function by suppressing macrophage and microglial activation by HIV in the central nervous system (CNS) have not yet been successful, in part, due to limited knowledge of the cellular mechanisms that control toxin secretion. Human monocytes, macrophages and microglia express the neurotrophin receptors p75 (p75NTR) and TrkA and respond differentially to nerve growth factor (NGF) and its precursor proNGF, by downregulating or exacerbating HIV-induced toxic activity, respectively. To determine how neurotrophins might influence HIV-associated activation, we evaluated the interaction between the neurotrophin receptors and the HIV co-receptor CXCR4. Blockade of CXCR4 but not CCR5 reduced HIV-induced neurotoxin secretion as assessed by calcium destabilization in primary rat cortical neurons. A similar suppression of HIV-induced neurotoxin secretion was seen when the p75NTR was blocked whereas blockade of TrkA signaling enhanced macrophage neurotoxin secretion. HIV stimulation suppressed calcium spiking in macrophages which was reversed by NGF but not proNGF. The reversal by NGF was dependent on CXCR4 since it was blocked by the specific antagonist AMD3100 and a CXCR4 neutralizing antibody. These results indicated that the neurotrophin receptors p75NTR and TrkA have opposing roles in macrophage neurotoxin production and may interact closely with HIV stimulation of CXCR4. Co-immunoprecipitation confirmed that CXCR4 formed complexes with

p75NTR and TrkA. NGF stimulation of macrophages in the presence or absence of HIV increased CXCR4 phosphorylation whereas HIV stimulation alone or proNGF had small or negligible effects. NGF induced phosphorylation was blocked by inhibition of TrkA signaling. Immunostaining showed that neurotrophin receptors and phosphorylated CXCR4 (pCXCR4) were expressed in similar cellular domains of the macrophages. HIV reduced the co-localization of pCXCR4 with both p75NTR and TrkA. NGF but not proNGF restored overlap between pCXCR4 and the p75NTR and partially restored pCXCR4/TrkA overlap. G-protein receptor kinase 2 (GRK2) was also associated with CXCR4, with a pattern that matched CXCR4 phosphorylation. This interaction between neurotrophin receptors and CXCR4 identifies a novel mechanism by which neurotrophins orchestrate phenotypic modifications of the macrophage and regulate inflammation in the central nervous system.

Disclosures: **V. Zhuravleva:** None. **K. Williams:** None. **J. Seawell:** None. **R.B. Meeker:** None.

Poster

047. HIV Neuroinflammation

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Program#/Poster#: 47.15/E7

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

Support: NIH Grant 5R01AG043540-02

Title: NeuroAIDS in NSG mice with humanized brain and hematolymphoid tissue

Authors: *W. LI, L. WU, J. KNIBBE, S. GORANTLA, H. E. GENDELMAN, L. Y. POLUEKTOVA;
Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Rodent models of human immunodeficiency virus (HIV) brain infections are limited as none reflect human glial-immunocyte-virus interactions as they occur in an infected human host. To achieve such a goal would require simultaneous humanization of both the immune system and brain. This was now achieved by combined reconstitution of the peripheral hematolymphoid and brain tissue. Herein, human glial precursors (GP) were generated from human fetal tissue. GP derived from neural stem cells were injected into lateral ventricle of neonatal NSG mice. The same mice were simultaneously inoculated intrahepatically with CD34+ hematopoietic stem cells (HSC) from human fetal liver from the same donor. At two months of age blood was tested for human immune cells by flow cytometry. Animals were infected with

HIV-1 peripherally by intraperitoneal inoculation then observed for 7 weeks and sacrificed. Brain tissues demonstrated anatomically symmetric humanization of both hemispheres by human glial cells. Immunohistochemistry for Glial fibrillary acidic protein and microtubule-associated protein 2 antigens showed the presence of large number of human astrocytes. In corpus callosum and periventricular areas, 70-90% of the identified astrocytes were of human origin. These cells were also distributed through the meninges and ventricular walls. Their presence did not induce mouse microglial activation. HIV-1 infection in these dual humanized mice showed blood viral load ranging from 1.55x105 to 6.14x105 copies/mL and HIV p24+ cells readily identified in mouse spleen. Virus infected mice brains showed reduced human glial cell density but increased Ki67+ nuclear staining. In contrast to HIV-1 infected animals without glial repopulation the dual reconstituted HIV infected mice showed increased peripheral lymphocytes and monocytes infiltration in the brains. Successful reconstitution of NSG mouse with both human glial cells and peripheral hematopoiesis offers a novel animal system to systematically explore the pathological mechanism underlying human-specific virus infections as they occur in an infected human host.

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Poster

047. HIV Neuroinflammation

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

Support: AA006420

AA020608

DA004398

MH062962

MH062512

CA121852

AA021667

Title: Gene expression changes consistent with neuroAIDS and impaired working memory in HIV-1 transgenic rats

Authors: *P. P. SANNA[1], C. LEFEBVRE[2], O. GEORGE[3], M. MORALES[4], G. F. KOOB[5], E. MASLIAH[6], A. CALIFANO[7], V. REPUNTE-CANONIGO[8];
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Abstract: We profiled gene expression in the hippocampus of HIV-1 Tg rats, a rodent model of HIV infection in which multiple HIV-1 proteins are expressed under the control of the viral LTR promoter in disease-relevant cells including microglia and astrocytes. The Gene Set Enrichment Analysis (GSEA) algorithm was used for pathway analysis. Gene expression changes observed are consistent with astrogliosis and microgliosis and include evidence of inflammation and cell proliferation. Among the genes with increased expression in HIV-1 Tg rats was the interferon stimulated gene 15 (ISG-15), which was previously shown to be increased in the cerebrospinal fluid (CSF) of HIV patients and to correlate with neuropsychological impairment and neuropathology, and prostaglandin D2 (PGD2) synthase (Ptgds), which has been associated with immune activation and the induction of astrogliosis and microgliosis. GSEA-based pathway analysis highlighted a broad dysregulation of genes involved in neuronal trophism and neurodegenerative disorders. Among the latter are genesets associated with Huntington's disease, Parkinson's disease, mitochondrial, peroxisome function, and synaptic trophism and plasticity, such as IGF, ErbB and netrin signaling and the PI3K signal transduction pathway, a mediator of neural plasticity and of a vast array of trophic signals. Additionally, gene expression analyses also show altered lipid metabolism and peroxisomes dysfunction. Supporting the functional significance of these gene expression alterations, HIV-1 Tg rats showed working memory impairments in spontaneous alternation behavior in the T-Maze, a paradigm sensitive to prefrontal cortex and hippocampal function. Comparison of gene expression of the hippocampus of HIV-1 Tg rats with the National NeuroAIDS Tissue Consortium (NNTC) human HIV gene expression dataset showed largely consistent results including changes in in genes related to neuronal trophism and degeneration. Altogether, differentially regulated genes and pathway analysis identify specific pathways that can be targeted therapeutically to increase trophic support, e.g. IGF, ErbB and netrin signaling, and reduce neuroinflammation, e.g. PGD2 synthesis, which may be beneficial in the treatment of chronic forms of HIV-associated neurocognitive disorders in the setting of viral suppression.g

Disclosures: **P.P. Sanna:** None. **C. Lefebvre:** None. **O. George:** None. **M. Morales:** None. **G.F. Koob:** None. **E. Masliah:** None. **A. Califano:** None. **V. Repunte-Canonigo:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.17/E9

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

Support: NIH Grant R21NS087406

NIH Grant T32ES019851

Title: Protective effects of the lignan secoisolariciresinol diglucoside (SDG) against oxidant stress, neuroinflammation, and HIV neurotoxicity

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Abstract: HIV-associated neurocognitive disorder (HAND) is a neurologic syndrome consisting of cognitive, motor, and behavioral deficits. Up to 50% of HIV-infected individuals are afflicted with HAND despite effective viral suppression with antiretroviral therapies (ART). Therefore, the identification of novel therapies to mitigate CNS complications of HAND would greatly improve the quality of life in HIV-infected patients. Such therapeutics would likely need to be brain-penetrant, and target multiple HAND-associated pathologies and be safe for prolonged use. Secoisolariciresinol diglucoside (SDG), a flaxseed-derived lignan, has anti-inflammatory and antioxidant properties and protects against damage to peripheral organ systems in diverse disease contexts. Therefore, we hypothesized that SDG could also protect against inflammation and oxidative stress in multiple CNS cell types, and protect neurons in an *in vitro* model of HIV neurotoxicity. Our data in mice demonstrate that SDG is CNS-penetrant and when administered systemically, acts as an indirect antioxidant in the CNS by activating the endogenous antioxidant response. *In vitro*, SDG protects against astrocyte loss following treatment of rat neuroglial cultures with hydrogen peroxide, and inhibits inflammatory responses in primary rat microglia induced by TNF-α. Finally, we show that SDG has protective effects against oxidative stress and neuronal loss in rat neuroglia treated with supernatants from HIV-infected human macrophages. Together, these data demonstrate that SDG has protective effects in multiple CNS cell types *in vitro*, and suggest that activities of SDG could synergize to mitigate inflammation and oxidative stress in HAND.

Disclosures: **M.A. Erickson:** None. **J. King:** None. **A. Odeleye:** None. **K. Seto:** None. **R. Pietrofesa:** None. **C. Akay Espinoza:** None. **B. Winkelstein:** None. **M. Christofidou-Solomidou:** None. **K. Jordan-Sciutto:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

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Program#/Poster#: 47.18/E10

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

Support: 1 SC1 GM113691-01

8G12MD007600

G12MD007579

R25GM061838

Title: Cathepsin B released from HIV-infected macrophages is internalized by neurons and induces apoptosis in rat primary cortical neurons

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Abstract: Human immunodeficiency virus (HIV-1) targets CD4-positive lymphocytes, macrophages and microglia. HIV-infected macrophages infiltrate trough the blood brain barrier into the brain, triggering a strong inflammation that results in synaptodendritic injury, neuronal dysfunction and apoptosis. Clinical manifestations range from asymptomatic, mild cognitive disorders to encephalitis and dementia. HIV-infected macrophages secrete cathepsin B, a lysosomal protease, inducing apoptosis of SK-N-SH neuroblastoma cells. The apoptosis is decreased by 10% when macrophage-conditioned medium is pre-treated with cathepsin B inhibitor or antibody. Cathepsin B is also increased in the deep frontal white matter of patients with HIV-associated dementia. The molecular action of cathepsin B remains unknown. We therefore hypothesize that HIV infection of macrophages fails to prevent cathepsin B internalization into neurons, promoting apoptosis. To determine the mechanism of cathepsin B-induced neuronal death, we exposed SK-N-SH to active recombinant His-tagged cathepsin B for

24 hours, and tracked its entry with a PE-conjugated His-tag antibody by intracellular flow cytometry. A high percentage of neurons internalized His-tagged cathepsin B diluted in plain medium (93.8%) or medium derived from HIV-infected macrophages (86.9%) when compared to neurons exposed to His-tagged cathepsin B in medium derived from uninfected macrophages (35.6%). Neuronal internalization of His-tagged cathepsin B was confirmed by western blot. We next determined the contribution of macrophage secreted cathepsin B to apoptosis in primary neurons. We exposed primary rat cortical neurons to uninfected and HIV-infected macrophage conditioned media pre-treated cathepsin B antibody for 24 hours and tested caspase-3 by western blot. Cleavage of caspase-3 is higher in neurons exposed to HIV-infected macrophage-conditioned medium compared to neurons exposed to uninfected macrophage medium. Moreover, pre-treatment of HIV-infected macrophage-conditioned medium with anti-cathepsin B antibody decreases the levels of cleaved caspase-3 in neurons, therefore decreasing apoptosis. In conclusion, we report for the first time that HIV-1 infection of macrophages fails to prevent internalization of cathepsin B by neurons and that macrophage-secreted cathepsin B induces apoptosis in primary neurons. Our results support the role of cathepsin B interference in reducing HIV-induced neuronal damage, representing a new approach for restoring the protective activity of macrophages against HIV-associated neurocognitive disorders.

Disclosures: Y.M. Cantres-Rosario: None. **M. Plaud:** None. **Y. Gerena:** None. **R.J. Noel:** None. **L.M. Melendez:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.19/E11

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

Title: Changes of mitochondrial homeostasis in hippocampus of hiv transgenic(tg26) mice

Authors: *P. R. GUDA[1], T. MAKAR[2,3], S. RAY[2], A. SAGI[2], J. BRYANT[4];
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Abstract: Mitochondrial physiology is fundamental to the maintenance of cellular function. This is particularly relevant in the nervous system, in which pathology is mostly associated with mitochondrial deficiencies. Defects in mitochondria are indeed key players in most neurodegenerative diseases, including HIV- associated dementia. There is no clear information

regarding mitochondrial roles in HIV associated dementia. The objectives of this study were to evaluate mitochondrial biogenesis, fusion/fission, mitochondrial function and apoptotic neuronal cell death in HIV-transgenic (Tg26) mice in relation to HIV progression. This was a hippocampal comparison study between five age and gender-matched groups (n=5×3) of healthy wild type (WT) mice and HIV-transgenic (Tg26) mice. We analyzed mitochondrial biogenesis, mitochondrial fusion and fission, mitochondrial apoptosis, and mitochondrial function by RT-PCR and Western blot analysis. Mitochondrial biogenesis (as measured by PGC-1α, Nrf1, Nrf2, and TFAM mRNA and protein levels) was decreased significantly in Tg26 mice compared to WT mice. Mitochondrial Fusion (as determined by Mfn-2 and OPA-1) was decreased in Tg26 mice with respect to WT mice. On the other hand, mitochondrial fission (as determined by Drp-1, Fish-1) was increased. All apoptotic parameters (Caspase-3, Caspase-9, and Bax) significantly increased. However, the mitochondrial functional parameter (COX-6) was inhibited in Tg26 mice compared to WT mice. Findings from this study identify a novel mitochondrial role in neuronal apoptosis associated with HIV induced neurocognitive disorder. Furthermore, we propose that this animal model is effective for mitochondrial and apoptotic assessment and could be useful for clinical research purposes.

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Poster

047. HIV Neuroinflammation

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

Support: NIH R01 DA034231

NIH R01 DA033200

Title: HIV-1 Tat-mediated neurotoxicity and anxiety-like behavior of mice may be protected by the pregnane neurosteroid, allopregnanolone

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Abstract: Human immunodeficiency virus (HIV) infection is associated with neurodegeneration and mood disorders. Gender/sex differences in the prevalence of such disorders are reported.

Actions of the HIV-1 regulatory protein, trans-activator of transcription (Tat), are involved in HIV-associated toxicity and may be partly ameliorated by sex steroid hormones. Using mice that conditionally-express HIV-1 Tat1-86 in the CNS, we have observed less behavioral impairment, neurotoxicity, astrogliosis, and neuroinflammation among Tat-exposed females compared to their male counterparts. Moreover, progesterone administration reduced anxiety-like impairments in ovariectomized transgenic female mice centrally-expressing Tat. We hypothesized that the Tat-protective effects of progesterone involved metabolism to its neuroprotective metabolite, allopregnanolone, given that allopregnanolone's protective actions overlap with targets of Tat-excitotoxicity. Complimentary *in vitro* and *in vivo* experiments were conducted. Neuronal co-cultures were incubated with media that did, or did not, contain allopregnanolone (100 nM) in the absence or presence of HIV-1 Tat1-86 (50 nM). Transgenic, female mice that did, or did not, express the transcription factor necessary for CNS-mediated Tat expression were ovariectomized and received a hormone-replacement regimen that consisted of intermittent progesterone (4 mg/kg), with or without pretreatment of a progesterone-metabolism inhibitor (finasteride 50 mg/kg). Mice were assessed in a battery of anxiety-like tasks (open field, elevated plus maze, marble burying). Consistent with prior reports, HIV-1 Tat caused significant neurotoxicity in culture and increased anxiety-like behavior of ovariectomized mice. Allopregnanolone significantly attenuated Tat's neurotoxic effects *in vitro* and progesterone ameliorated anxiety-like behavior associated with central Tat expression *in vivo*. The latter protection could be blocked via pretreatment with finasteride. Thus, pregnane steroids confer neuroprotection *in vitro* and ameliorate anxiety-like impairments *in vivo* that are associated with HIV-1 Tat. These effects may be dependent on formation of the neurosteroid, allopregnanolone. Hormone therapies may confer prophylactic advantages in the treatment of neuroAIDS.

Disclosures: **J.J. Paris:** None. **D. Chen:** None. **S. Kim:** None. **P.E. Knapp:** None. **K.F. Hauser:** None.

Poster

047. HIV Neuroinflammation

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Program#/Poster#: 47.21/E13

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

Support: USPHSG DA033206

USPHSG DA033882

P30AI082151

Title: Enhanced expression of immunoreactive L-type calcium channels in the mesocorticolimbic pathway of HIV-1 transgenic rats

Authors: *W. N. WAYMAN, A. L. PERSONS, T. C. NAPIER;
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Abstract: The mesocorticolimbic pathway is affected in HIV^+ humans by toxic HIV-1 proteins. Two key structures of the mesocorticolimbic system are the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc). Recently, we revealed that the pathophysiology occurs in mPFC pyramidal neurons and NAc medium spiny neurons from HIV-1 transgenic (Tg) F344 rats as compared to non-transgenic F344 controls (non-Tg) (Wayman et al. JNIP 10:S108, 2015; Chen et al. JNIP 10:S64, 2015). There were striking differences in the electrophysiological profiles of mPFC pyramidal neurons and NAc medium spiny neurons between Tg and non-Tg rats. Both pyramidal and medium spiny neurons from Tg rats demonstrated hyper-excitability, including increased evoked and spontaneous firing, and a greater tendency for depolarization-induced inactivation. In the mPFC, this altered electrophysiological profile was mitigated by diltiazem, indicating a contribution of high-voltage-activated L-type Ca^{2+} channels. Here, we extend this work by determining if expression of $Ca_{v1.2}$-α1c (the pore forming subunit of L-type Ca^{2+} channels) is altered in male, young adult Tg rats. Brain tissue from non-Tg and Tg rats was harvested and prepared for immunohistochemical assessments of $Ca_{v1.2}$-α1c. We found that $Ca_{v1.2}$-immunoreactivity (ir) was readily detected in the mPFC and NAc of both non-Tg and Tg rats. For these pilot studies, observers who were blinded to genotype, qualitatively determined the staining patterns of $Ca_{v1.2}$-ir of neurons in mPFC layers 5/6 and the NAc core. Staining scores ranged from 1, which described a low number of $Ca_{v1.2}$-ir neurons with low staining intensity, up 4, which described a high number of $Ca_{v1.2}$-ir neurons with high staining intensity. In layers 5/6 of the prelimbic mPFC revealed differences in staining for $Ca_{v1.2}$-ir between non-Tg and Tg rats. The average score for $Ca_{v1.2}$-ir in the mPFC of Tg rats was 3 compared to score of 2 for the $Ca_{v1.2}$-ir in non-Tg rats Likewise, the average score for $Ca_{v1.2}$-ir in the NAc core of Tg rats was 4, compared to a score of 2 for the $Ca_{v1.2}$-ir in non-Tg rats. Taken together, these findings indicate increased expression of L-type Ca^{2+} channels may contribute to the increased neuronal excitability observed in Tg rats.

Disclosures: **W.N. Wayman:** None. **A.L. Persons:** None. **T.C. Napier:** None.

Poster

047. HIV Neuroinflammation

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

Support: NIH/NIMH R01-MH087332

NIH/NIMH R01-MH104131

Title: IFNβ protects cerebrocortical neurons in a CCL4-dependent fashion against HIV-1 gp120-induced injury

Authors: *V. E. THANEY[1], M. M. HOEFER[1], M. KAUL[1,2];
[1]Infectious and Inflammatory Dis. Ctr., Sanford Burnham Med. Res. Inst., La Jolla, CA; [2]Dept. of Psychiatry, Univ. of California San Diego, San Diego, CA

Abstract: HIV-1 appears to invade the CNS soon after peripheral infection. However, severe neurological symptoms may be delayed until a later stage of disease progression. Type I interferons are critical mediators of anti-viral immune response and IFNβ has been implicated in the control of HIV and SIV infection of the brain. However, the potential role of IFNβ as a neuroprotective factor in the context of HIV/gp120-induced neuronal injury remains to be characterized. Therefore, we investigated the neuroprotective potential of IFNβ against toxicity of HIV/gp120 in the brain using *in vivo* and *in vitro* models. Using mixed rodent cerebrocortical cultures (RCC), containing neurons, astrocytes, and microglia, we found that treatment with IFNβ can provide concentration-dependent neuroprotection against HIV/gp120. Additionally, treatment with IFNβ of RCC increased levels of natural ligands of the HIV co-receptor CCR5 and up-regulated expression of anti-viral IFN-stimulated genes (ISGs). These ligands, MIP-1β/CCL4 and RANTES/CCL5, are known to suppress HIV-1 infection; disease progression and all can provide significant *in vitro* protection against gp120-induced neuronal injury. In fact, we found that MIP-1β/CCL4 is required for IFNβ mediated neuroprotection. Recently, we used a microarray-based genome-wide gene expression analysis to identify genes that are differentially expressed in connection with the neuropathological phenotype of HIV/gp120-transgenic (tg) mice at different ages. HIVgp120tg mice express the viral envelope protein in the brain and manifest several neuropathological features observed in AIDS brains, such as decreased synaptic and dendritic density, increased numbers of activated microglia, and pronounced astrocytosis. We found that gp120tg brains can mount an anti-viral immune response, specifically inducing the expression of ISGs. In addition, qRT-PCR analysis confirmed a transient IFNβ expression in gp120tg brains and up-regulation of numerous ISGs, prior to the development of neuropathology and behavioral impairment. Moreover, a four-week intranasal IFNβ treatment completely abrogated neuronal damage in gp120tg mice while triggering a biological response of IFN-induced gene expression. Similarly to the *in vitro* studies, we found that IFNβ treatment significantly increased MIP-1β/CCL4 expression levels in the HIV/gp120tg brains and therefore propose a critical role for this chemokine in the mechanism of IFNβ mediated neuroprotection.

Disclosures: **V.E. Thaney:** None. **M.M. Hoefer:** None. **M. Kaul:** None.

Poster

047. HIV Neuroinflammation

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

Support: NIH Grant 1P20GM103643

NIH Grant 5R21NS066130

Title: M1/M2 polarization and type 1 interferon response in morphine-potentiated LP-BM5 murine AIDS

Authors: *V. D. MCLANE[1,2], C. L. WILLIS[1], L. CAO[1];
[1]Col. of Osteo. Med., Univ. of New England, Biddeford, ME; [2]Grad. Sch. of Biomed. Sci. and Engin., Univ. of Maine, Orono, ME

Abstract: Of the 1.1 million people infected with human immunodeficiency virus (HIV-1) in the United States, it is estimated that between 15 and 50% will develop cognitive deficits. Over 30% of HIV-1 patients abuse opiates such as heroin, which increase the risk and severity of HIV-associated neurocognitive disorders. Morphine mediates this effect through its influence on glia, the initiators of innate immune defense against viral infection in the central nervous system (CNS). Through the LP-BM5 murine acquired immunodeficiency syndrome (MAIDS) model, we have shown that morphine induces region-specific changes in viral load and cytokine expression in the CNS. We hypothesized that the change in viral RNA is the result of the synergistic effects of morphine and LP-BM5 viral infection on the antiviral type 1 interferon response and the proinflammatory cytokine response of the CNS. To investigate this, we infected male C57BL/6 mice with LP-BM5 (5e4 plaque-forming units, intraperitoneal injection). At 7 weeks post-infection, animals received 1 week of treatment with a subcutaneous morphine (25 mg) or placebo pellet. We measured expression of type 1 interferons (IFN-α and IFN-β) and pro- (M1) and anti-inflammatory (M2) markers in key regions of interest - hippocampus, striatum, and frontal cortex - through quantitative real-time PCR and immunohistochemistry. Following morphine treatment, we observed reduced viral load and significantly higher type 1 IFN relative expression in striatum compared to the hippocampus, which exhibits increased viral load and a weak type 1 IFN response. We hypothesize that the reduced type 1 IFN response, in conjunction with the global reduction in proinflammatory cytokines such as CCL5, leaves the hippocampus vulnerable to viral infection. Through our work with the LP-BM5/MAIDS model, we are

identifying novel pathways for the treatment and prevention of opiate-potentiated HIV-associated neurocognitive disorders.

Disclosures: **V.D. McLane:** None. **C.L. Willis:** None. **L. Cao:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

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Program#/Poster#: 47.24/E16

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

Support: NIH Grant R01 CA021776

Title: Pharmacologic targeting of host innate immune responses decreases viral replication in a brain slice culture model of HSV encephalitis

Authors: ***D. R. WILCOX**[1,2], W. J. MULLER[1], R. LONGNECKER[2];
[1]Pediatrics, [2]Microbiology-Immunology, Northwestern Univ. Sch. of Med., Chicago, IL

Abstract: The increased susceptibility and severity of herpes simplex virus (HSV) infection in the newborn compared to the adult is thought to be due to differences in the host response to infection between the developing and the mature brain, but the precise reasons remain unknown. In order to successfully replicate in the host cell, HSV counters several pathways in the innate immune response to infection. Our group and others have demonstrated that the multifunctional HSV protein γ34.5 counters several pathways in the host response to infection, and contributes to disease in both the newborn and adult CNS. Although HSV inhibition of autophagy contributes to disease in the adult CNS, we previously demonstrated that it is dispensable for pathogenesis in the neonatal brain. We hypothesized that additional virus-host interactions mediated by HSV γ34.5 contributed to disease in the newborn. To investigate additional host interactions with HSV γ34.5 that contribute to CNS disease, we used a virus carrying a mutation that specifically disrupts γ34.5 binding to the host phosphatase PP1α (ΔPP1α). Intracranial inoculation of mice was used as a model of HSV encephalitis. In both newborn and adult mice, infection with ΔPP1α resulted in significant attenuation of the virus in the CNS, with a longer time to mortality and slower replication compared to the rescue virus. Furthermore, after inoculation of interferon-receptor knock out (IFNAR KO) mice, we show that attenuation of the ΔPP1α virus is IFN dependent in both age groups. To gain a detailed understanding of viral replication dynamics and specific host targets of PP1α modulation by HSV, we established an *ex vivo* organotypic brain slice culture (BSC) model of HSV encephalitis. In BSCs from either newborn or adult mice, the

ΔPP1α virus replicated more slowly than the rescue virus control. HSV γ34.5 binding of PP1α resulted in retargeting of the host phosphatase to dephosphorylate eIF2α, effectively reversing translational arrest in the host cell during infection. Furthermore, treating BSCs with salubrinal, a drug that causes the phosphorylation of eIF2α, significantly reduces viral replication in both the adult and newborn brain. We conclude from our data that reversal of host translational arrest by HSV binding of PP1α contributes to CNS pathogenesis in both age groups, and salubrinal, a drug that increases eIF2α phosphorylation, significantly decreases viral replication in a BSC model of HSV encephalitis.

Disclosures: D.R. Wilcox: None. **W.J. Muller:** None. **R. Longnecker:** None.

Poster

047. HIV Neuroinflammation

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.25/E17

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

Support: F31 NS086256-01

Title: Modeling HIV-1-induced platelet-mediated dysfunction of the blood-brain barrier in mice

Authors: *L. JONES[1], V. SINGH[1], D. DAVIDSON[2], S. MAGGIRWAR[1];
[1]MBI, Univ. of Rochester Med. Ctr., Rochester, NY; [2]Pathology and Physiol. Res. Br., Natl. Inst. for Occup. Safety and Hlth., Morgantwon, NY

Abstract: In spite of combined antiretroviral therapy (cART), the number of HIV-1 positive individuals developing some form of HIV-associated neurocognitive disorder (HAND) is increasing. Normally, the blood-brain barrier (BBB) serves to regulate transport into and out of the CNS, thus serving as a protective barrier; however, in HIV-1infected individuals, the integrity of the BBB is compromised due to an increase in the expression of proinflammatory mediators as well as viral proteins. Our lab, and others, has shown that sCD40L is released upon platelet activation and important mediator of the pathogenesis of HAND. However, the molecular mechanisms underlying this phenomenon are not fully understood. In order to recapitulate the factors observed in HIV-1 positive individuals, and thus identify the key mechanisms that drive the pathogenesis of HAND, an animal model is needed. Consistently, in our study we have utilized a novel animal model in which wild-type (WT) mice are infected with EcoHIV; a virus with the HIV-1 genomic gp120 attachment protein replaced with MuLV's gp80. We hypothesized that EcoHIV would replicate efficiently *in vivo* and would be able to induce

pathology similar to that observed in HIV-1-infected humans. Here we now demonstrate that upon infection with EcoHIV, platelets are activated, and as early as two-weeks post infection, BBB permeability is increased. In addition, tight junction proteins of the CNS are adversely affected. Here in, the results are evidence that platelet activation and sCD40L are still mediators of BBB disruption in a model of chronic inflammation. Therefore, this animal model will serve as a stepping-stone toward expanding research into the molecular mechanisms underlying HAND, and underscores the utility of this model to study further therapeutic treatments.

Disclosures: L. Jones: None. **V. Singh:** None. **D. Davidson:** None. **S. Maggirwar:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.26/E18

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

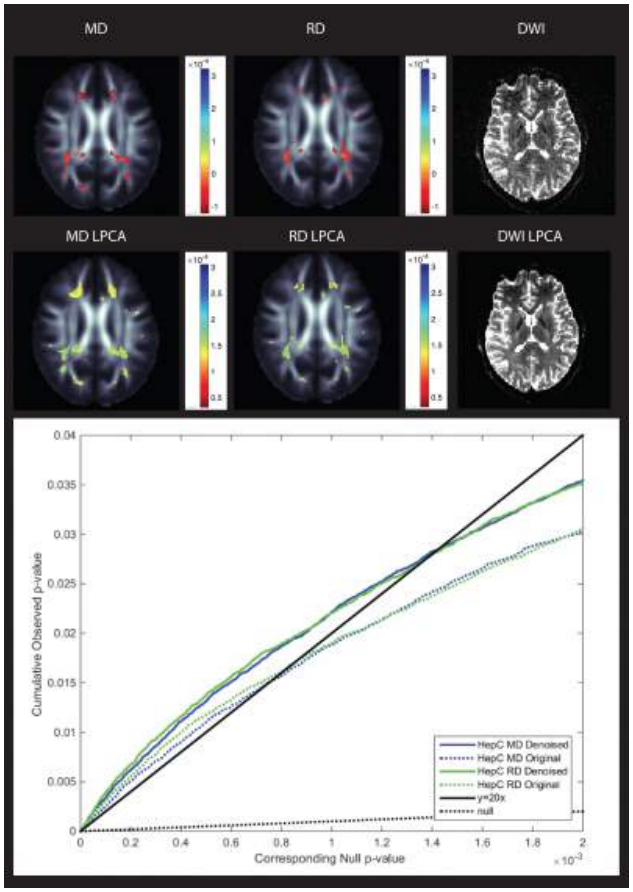
Support: R01 MH074368/MH/NIMH NIH HHS/United States

Title: Denoising of diffusion MRI boosts power to detect Hepatitis C effects on the brain in HIV+ adults

Authors: *D. SCHONFELD[1], T. M. NIR[1], N. JAHANSHAD[1], C. R. K. CHING[1], X. HUA[1], A. GONGVATANA[2], B. NAVIA[3,4], R. A. COHEN[5,6], P. M. THOMPSON[1];
[1]Imaging Genet. Center/Keck Sch. of Med. of, Los Angeles, CA; [2]UCSD, San Diego, CA; [3]Tufts Univ. Sch. of Med., Boston, MA; [4]Dept. of Publ. Heath and Community Med., Tufts Sch. of Med., Boston, MA; [5]Dept. of Aging-Geriatric Res., Univ. of Florida Col. of Med., Gainesville, FL; [6]Brown Univ. Sch. of Med., Providence, RI

Abstract: Hepatitis C may affect white matter integrity, especially in the context of co-morbid HIV infection. Here we used diffusion tensor imaging (DTI) to detect effects of Hepatitis C virus (HCV) on white matter (WM) integrity in an adult HIV+ population. We also tested whether effects were more sensitively detected after we incorporate a local PCA denoising approach (Manjon et al., 2013) into our image analysis workflow. 62 HIV+ patients (age: 45.25 +/- 10.38 yrs ; 41 M/21 F) were scanned with 3D volumetric T1-weighted anatomical and diffusion MRI (10 b0 and 64 DWI). 23 patients had co-morbid HCV infection. Both raw and denoised sets of the data were preprocessed in parallel. Fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) maps were calculated from the diffusion tensor after correcting for eddy current and EPI distortions. Each subject's FA map was then elastically

registered to a study-specific minimal deformation template (MDT), based on FA, and the warps were then applied to all diffusivity maps. Voxel-wise linear regressions, adjusted for sex and age, tested for the effects of HCV co-infection on DTI measures of WM integrity. We corrected for multiple comparisons using the false discovery rate (FDR) procedure. Subjects co-infected with Hepatitis C had significantly higher MD than the HIV mono-infected group (FDR critical raw $p < 0.00075$; LPCA $p < 0.0014$) and RD (FDR critical raw $p < 0.00086$; LPCA $p < 0.0013$) - a sign of poorer WM integrity. The image denoising method detected twice as many significant voxels after FDR correction, as shown in the cumulative distribution function (CDF) plots of the voxelwise *p*-value maps and the higher critical FDR p values (see Figure). Denoising DWI images may boost power to detect subtle brain differences associated with co-infection of HCV and HIV.



Disclosures: **D. Schonfeld:** None. **T.M. Nir:** None. **N. Jahanshad:** None. **C.R.K. Ching:** None. **X. Hua:** None. **A. Gongvatana:** None. **B. Navia:** None. **R.A. Cohen:** None. **P.M. Thompson:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.27/E19

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

Support: National Natural Science Foundation of China, 81171134 and 81471235

Title: Effect of autophagy on L-type calcium channel current induced by gp120V3 loop in hippocampus neurons

Authors: *J. DONG[1], Y. XING[2], Q. YU[4], J. WANG[2], G. CHEN[2], M.-L. JIANG[2], L. LIN[2], S. LIU[2], Y. XU[3];
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Abstract: This research aims to observe the influence of autophagy on L-type calcium channel current induced by gp120V3 loop in hippocampal neurons. Hippocampal neurons were exteriorized from rats born within one day and then cultured for seven days before they were used for experiments. Experiments were divided into two parallel groups, namely control group, gp120V3 loop group, autophagy inhibotor 3-MA group, gp120V3 loop plus 3-MA group; control group, gp120V3 loop group, autophagy activator Rapamycin group, gp120V3 loop plus Rapamycin group. Whole-cell patch clamp was used to record L-type calcium channel current. Compared with control group, L-type calcium channel current density significantly increased in gp120V3 loop group and 3-MA group ($P < 0.05$). Compared with gp120V3 loop group, L-type calcium channel current density significantly increased in gp120V3 loop + 3-MA group ($P <0.05$). Compared with control group, L-type calcium channel current density significantly decreased in Rapamycin group ($P <0.05$). Compared with gp120V3 group, L - type calcium channel current density significantly increased in gp120V3 + Rapamycin group ($P< 0.05$). Thus our electrophysiological data indicates that autophagy may be involved in the gp120V3 loop-mediated L-type calcium channels in hippocampus neurons.

Disclosures: J. Dong: None. **Y. Xing:** None. **Q. Yu:** None. **J. Wang:** None. **G. Chen:** None. **M. Jiang:** None. **L. Lin:** None. **S. Liu:** None. **Y. Xu:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.28/E20

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

Support: NIH Intramural Research Program

Title: Cognitive impairment in HIV: Assessment using manual, saccadic, and traditional neuropsychological measures

Authors: *T. SHIRAZI[1], A. SUMMERS[1], S. STEINBACH[2], B. SMITH[2], S. KAPETANOVIC[1], A. NATH[2], M. ETTENHOFER[3], J. SNOW[1];
[1]NIMH, Bethesda, MD; [2]NINDS, Bethesda, MD; [3]Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: Whereas some studies estimate the rate of cognitive impairment in HIV to be as high as 60%, other studies suggest no differences in cognitive function between HIV+ and HIV- individuals. Conflicting results have emerged in studies using traditional neuropsychological (NP) measures, considered to be the 'gold standard' in the evaluation of cognition, as well as in studies using measures of manual reaction time (RT), which allow for detection of more subtle cognitive deficits. However, saccadic measures, which have been evaluated in the context of cognition in various neurodegenerative disorders, have not been recently studied in HIV. Unlike NP and manual RT measures, saccadic measures may be more resistant to confounds of testing such as poor effort, depression, or intelligence. We compared cognitive function in HIV+ participants (n=15) and demographically matched HIV- controls (n=11) using manual and saccadic measures of RT and inhibition, as well as traditional NP tests. All HIV+ participants were on antiretroviral therapy (ART) and had well-controlled viremia. Participants were on average 50.25 years old (SD=9.75) and had 14.00 years of education (SD=2.57). Participants were administered a NP battery assessing 7 cognitive domains purported to be impaired in HIV, as well as a computerized task measuring manual and saccadic RT and inhibition. In the task, a white or red cue appeared at the center of the screen, and was followed by a white circle appearing either below, above, to the left, or to the right of the cue. Participants were instructed to make a saccade to the circle as well as manually press a button if a white cue preceded presentation of the circle, and to inhibit saccadic and manual responses if a red cue preceded presentation of the circle (go no-go task) while eye movements were recorded using remote optics. Data were analyzed using Applied Science Laboratories software, a customized data parser, and SPSS. There were no significant differences between HIV+ and HIV- participants in any of the 15 NP variables or on composite NP measures. Similarly, there were no significant between-group differences in manual RT, manual response inhibition, saccadic RT, and saccadic response inhibition (all $p>0.05$). Effect sizes were small (Cohen's d <0.4), with the exception of that for manual inhibition (d=0.46) and verbal delayed recall (d= 0.56). Taken together, the preliminary results suggest the absence of large differences in cognition between HIV+ and HIV-

participants in our small sample. Ability to detect subtle deficits could be increased by examining more specific saccadic and manual metrics, or more sophisticated diagnostic classification algorithms.

Disclosures: **T. Shirazi:** None. **A. Summers:** None. **S. Steinbach:** None. **B. Smith:** None. **S. Kapetanovic:** None. **A. Nath:** None. **M. Ettenhofer:** None. **J. Snow:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.29/E21

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

Title: Wnt7a skews monocyte differentiation: relevance to neuroAIDS

Authors: *J. WALLACE[1], L. AL-HARTHI[2];
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Abstract: Monocytes infiltrate the central nervous system for pathological and/or homeostatic purposes and differentiate into proinflammatory M-1, alternative M-2-like macrophages or a variation of intermediate phenotypes. The non-classical CD14+CD16+ monocytes have been observed to more readily traverse the blood brain barrier, and in HIV infection, an expansion of this phenotype correlates with worsened disease prognosis. Increased CD163 expression on brain macrophages/microglia is also associated with HIV encephalitic lesions. Published data from our lab illustrates that β-catenin; a protein primarily regulated by a family of morphogenetic glycoproteins known as Wnts, is a restrictive factor for productive HIV infection of monocytes. Canonical Wnt signaling stabilizes β-catenin, enabling its translocation to the nucleus, where it is a transcriptional co-activator. Non-canonical Wnts activate the Ca2+ and planar cell polarity (PCP) signaling pathways. Our objective is to evaluate the impact of Wnts on monocyte differentiation, phenotype and function as it relates to HIV neuropathogenesis. We show that human monocytes express all 19 Wnt mRNAs, and after 24 hours in culture, all but Wnts 2b, 9a, 10a, 10b and 16 are differentially expressed. This differential expression occurs in untreated monocytes as well as monocytes cultured in the presence of M-CSF or GM-CSF. Immunohistochemical staining for Wnts 1, 3 and 7a in C57BL/6 mouse brain slices revealed robust expression of Wnt 7a in the striatum and hippocampus. Additionally, culturing monocytes for 24 hrs with Wnts 1, 5a, or 7a recombinant proteins revealed that only Wnt 7a inhibited CD14+CD16- phenotype by 3-, 5- and 7- folds relative to untreated, M-CSF and GM-CSF treated groups respectively. After 7 days of Wnt 7a treatment, further inhibition of CD14+CD16-

(by 10- folds for both untreated and M-CSF treated and 25- folds relative to GM-CSF treated), CD14+CD16+ (3-, 5- and 12- folds for untreated, M-CSF treated and GM-CSF treated respectively), as well as CD163 expression (3-, 4-, and 7- folds for untreated, M-CSF and GM-CSF treated respectively) were measured. These Wnt 7A-mediated effects did not correlate with changes in active β- catenin expression. These data suggest that Wnt 7A induces monocyte differentiation, as indicated by down regulation of CD14. Ongoing studies are assessing the involvement of non-canonical Wnt pathways, as well as the phenotype and functionality of the MDMs from each experimental group. Ultimately, our studies will provide a better understanding of how Wnts within the CNS skew differentiation and function of infiltrating monocytes.

Disclosures: J. Wallace: None. **L. Al-Harthi:** None.

Poster

047. HIV Neuroinflammation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 47.30/E22

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

Support: NIH U54 EB020403

Title: High-resolution shape analysis in HIV+ adults reveals associations between neurocognitive performance and subcortical morphometry

Authors: *C. CHING[1,2], B. GUTMAN[2], T. NIR[2], D. SCHONFELD[2], N. JAHANSHAD[2], X. HUA[2], A. GONGVATANA[3], B. NAVIA[4], R. COHEN[5], P. THOMPSON[2];
[1]UCLA, Los Angeles, CA; [2]Imaging Genet. Center, Inst. for Neuroimaging & Informatics, Univ. of Southern California, Los Angeles, CA; [3]Dept. of Psychiatry, Univ. of California, San Diego, San Diego, CA; [4]Dept. of Publ. Health, Infection Unit, Tufts Univ. Sch. of Med., Boston, MA; [5]Dept. of Aging and Geriatric Research, Univ. of Florida, Gainesville, Gainesville, FL

Abstract: Up to 50% of HIV+ individuals experience neurocognitive impairment. Here we applied a novel subcortical shape analysis technique to better understand how impairment relates to brain structure in a large cohort of HIV+ adults. We hypothesized that shape analysis would detect subcortical morphometric differences associated with neurocognitive performance. T1-weighted brain MRI scans from 82 HIV+ patients were collected through the HIV-associated brain dysfunction study at Brown University (mean age: 45.6 +/- 9.9 yrs.; 54M/28F). Two shape metrics of local thickness and surface area were derived from structural MRI, radial distance

(thickness) and the Jacobian determinant (Jacobian) or surface dilation ratio to a template, across thousands of homologous points for the left and right nucleus accumbens, amygdala, caudate, hippocampus, putamen, pallidum, and thalamus shape models. A multiple linear regression was fit to predict thickness and Jacobian values at each surface point to evaluate associations with neurocognitive domain-specific T-scores where lower scores indicated greater impairment. Domains of interest included processing speed (PS), learning (LT), and motor function (MT). Analyses were adjusted for age, sex, ethnicity, intracranial volume, HIV duration, HIV RNA presence, history of Hepatitis C, antiretroviral treatment, history of drug use (alcohol, cocaine and opiates), and CD4+ T-lymphocyte count. All results were corrected for multiple comparisons using a standard FDR correction (q=0.05). Lower PS was significantly associated with lower Jacobian and thickness values in the left hippocampus/accumbens and right hippocampus/thalamus (Figure 1). Lower MT was associated with lower thickness values in the right pallidum. Lower LT was associated with lower Jacobian values in the left thalamus. High-resolution shape analysis revealed significant associations between reductions in subcortical volume/surface area and reduced processing speed, learning and motor function and may be a powerful new technique to track HIV+ related brain change.

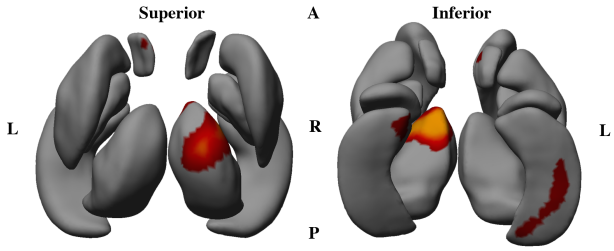

Figure 1: Associations between subcortical Jacobian values and processing speed (PS) showing β values for regions passing FDR correction (q=0.05). Red/Yellow regions indicate positive β values or regions where lower Jacobian values (reduced surface area and volume) are associated with lower PS scores (greater impairment). *Left Image: superior view; Right Image: inferior view; A: anterior; P: posterior; L: left; R: right*

Disclosures: **C. Ching:** None. **B. Gutman:** None. **T. Nir:** None. **D. Schonfeld:** None. **N. Jahanshad:** None. **X. Hua:** None. **A. Gongvatana:** None. **B. Navia:** None. **R. Cohen:** None. **P. Thompson:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.01/E23

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

Support: NIH Grant R01 MH091130

NIH Grant R25 GM095480

NIH Grant F31 MH105166

NIH Grant F31 MH098564

Title: Adolescent cannabinoid exposure increases the susceptibility for a schizophrenia-like phenotype in a novel rodent model

Authors: *D. D. AGUILAR, S. M. PEREZ, A. GIUFFRIDA, D. J. LODGE;
Pharmacol., UTHSCSA, San Antonio, TX

Abstract: Schizophrenia is a debilitating disorder that affects over one percent of the population. Adolescent cannabis abuse is associated with an increased risk of developing schizophrenia. However, this is not a causal relationship. As many adolescents use cannabis in a recreational setting and do not develop psychiatric illnesses, it is likely that cannabinoids increase the risk of developing schizophrenia only in individuals with an underlying predisposition. To explore this phenomenon, we have developed a model of "susceptibility" to schizophrenia-like symptoms. Specifically, the second filial (F2) generation of a developmental rodent model of schizophrenia [i.e. gestational methylazoxymethanol acetate (MAM) administration in rats] show increased susceptibility to a schizophrenia-like phenotype. We believe that this "F2 MAM" model is unique in that it provides a model of susceptibility by which we can examine potential gene x environment interactions as they pertain to schizophrenia. Based on the relationship between cannabinoids and schizophrenia, we hypothesized that cannabinoid exposure during adolescence would increase the proportion of 'susceptible' F2 generation MAM rats displaying a schizophrenia-like phenotype while having no observable effects in control animals. Our preliminary data shows that exposure to a high dose of the synthetic cannabinoid WIN55,212-2 (1.2mg/kg) during adolescence increases a schizophrenia-like phenotype in F2 MAM and control rats. However, a lower dose of WIN55,212-2 (0.2mg/kg) during adolescence seems to affect the more 'susceptible' F2 MAM but not control rats. Although exogenous cannabinoids can transiently evoke psychotic symptoms in adult patients with schizophrenia, endogenous cannabinoid upregulation can alleviate positive and negative symptoms in these patients. Rodent models of schizophrenia also experience therapeutic effects following endogenous cannabinoid upregulation in adulthood. Therefore, we were interested in the differences between adolescent exposure to endogenous and exogenous cannabinoids in our susceptible model. Augmenting endogenous cannabinoids during adolescence increased the proportion of F2 MAM rats displaying a schizophrenia-like phenotype without producing noticeable effects in control rats. This supported our theory that the F2 MAM population may be more susceptible to certain environmental factors during adolescence than controls.

Disclosures: D.D. Aguilar: None. **S.M. Perez:** None. **A. Giuffrida:** None. **D.J. Lodge:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.02/E24

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

Support: Grants-in-aid for Young Scientists (B) (26870878)

SENSHIN Medical Research Foundation

Research Group for Schizophrenia

Program for Advancing Strategic International Networks to Accelerate the Circulation of Talented Researchers (S2603)

Title: Telomere shortening in the hippocampus is associated with the negative symptoms of schizophrenia

Authors: *K. TORIUMI[1], M. MIYASHITA[1,2], T. ICHIKAWA[3], A. KOBORI[1], Y. HORIUCHI[1], M. ARAI[1], I. NOHARA[1], N. OBATA[1], H. HASHIMOTO[4,5,6], M. ITOKAWA[1], M. ARAI[1];
[1]Schizophrenia Res. Project, Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [2]Dept. of Psychiatry, Sch. of Med., Shinshu Univ., Matsumoto, Japan; [3]Dept. of Microbial Sci. and Host Def., Meiji Pharmaceut. Univ., Tokyo, Japan; [4]Lab. of Mol. Neuropharmacology, Grad. Sch. of Pharmaceut. Sci., [5]Mol. Res. Ctr. for Children's Mental Development, United Grad. Sch. of Child Develop., [6]iPS Cell-based Res. Proj. on Brain Neuropharmacol. and Toxicology, Grad. Sch. of Pharmaceut. Sci., Osaka Univ., Osaka, Japan

Abstract: Telomeres are tandem repeats located at the end of chromosomes, and they play a role in maintaining chromosomal integrity during cell division. It has been reported recently that leukocyte telomeres in patients with schizophrenia were shorter than those in healthy subjects. In addition, numerous studies have shown that telomere shortening can be induced by stress exposure in early life, which is a prominent risk factor for developing schizophrenia. However, the molecular link between telomere shortening and schizophrenic pathophysiology is still unclear. We detected the interesting connection among telomere shortening in the hippocampus, stress exposure and schizophrenia. We found that the therapeutic effects of second-generation antipsychotics might be mediated by hippocampal telomerase activation via the antagonism of serotonin 5-HT2A receptors, and telomere shortening in hippocampus might be related to the negative symptom-like behavior, cognitive impairment, and prepulse inhibition deficit, but not to

the positive symptom-like behavior in schizophrenia. They are novel findings of telomere alteration that connects a specific brain region, a molecular pathway, and the associated behavioral phenotypes using human samples and a mouse model. Our report suggests that drugs modulating telomeres might serve as novel medications for the treatment of schizophrenia.

Disclosures: K. Toriumi: None. **M. Miyashita:** None. **T. Ichikawa:** None. **A. Kobori:** None. **Y. Horiuchi:** None. **M. Arai:** None. **I. Nohara:** None. **N. Obata:** None. **H. Hashimoto:** None. **M. Itokawa:** None. **M. Arai:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.03/E25

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

Title: Determination of mitochondrial activity in a mice juvenile two-hit model of schizophrenia

Authors: *C. MONPAYS[1], J. DESLAURIERS[2], P. SARRET[2], S. GRIGNON[2,3];
[1]Physiol. and Biophysics, [2]Univ. De Sherbrooke, Sherbrooke, QC, Canada; [3]Psychiatry, Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: Schizophrenia is a chronic mental illness characterized by different clinical symptoms with three core features (positive (eg hallucinations), negative (eg lack of motivation) and cognitive (eg executive dysfunction). Among a large array of neurochemical disturbances, imbalance between production of reactive oxygen species and activity of antioxidant enzymes, convincingly points toward mitochondrial dysfunction. Our laboratory has recently developed a juvenile murine two-hit model (THM) of schizophrenia based on the combination of two environmental risk factors (gestational inflammation induced by poly IC, followed by juvenile restraint stress at postnatal days 33-35) to gain a better understanding of the early disease onset. We previously reported relevant behavioral and neurochemical disturbances, including oxidative stress (as assessed by an increase in protein carbonylation), thus providing preliminary validation of this THM of schizophrenia. Moreover, the antioxidant lipoic acid (LA) reversed these deficits, thereby pointing to a key role of oxidative stress in schizophrenia. Here, we investigated mitochondrial function in this juvenile murine THM of schizophrenia. The mitochondrial activity was determined using the Mitoxpress commercial kit within two relevant regions (prefrontal cortex (PFC) and striatum) associated with schizophrenia. Our measures were performed in state 3, with substrates for complex I (glutamate-malate + ADP) and complex II (succinate + ADP) induced mitochondrial respiratory activity. We observed an increase in complex I induced

respiratory activity in the PFC and striatum in both sexes but an increase in complex II activity only in males. LA treatment prevented this increase only in complex II induced respiration in males but had no effect on complex I induced activity. Expression levels of the different respiratory chain complexes were not modified under our conditions. In conclusion, our juvenile two-hit model of schizophrenia shows an increase in mitochondrial activity reversed by lipoic acid treatment, specifically in complex II induced respiratory activity in males, without changes in respiratory chain protein expression. Further work is required to investigate the origin and consequences of these modifications.

Disclosures: **C. Monpays:** None. **J. Deslauriers:** None. **P. Sarret:** None. **S. Grignon:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.04/E26

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

Support: MH57440

10865/13-7, CAPES-Brazil

Title: Adolescent cannabinoid administration negatively impacts the dopaminergic system and cognition in normal rats, but provides a protective effect in MAM schizophrenia model

Authors: *F. V. GOMES[1], F. S. GUIMARAES[2], A. A. GRACE[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Med. Sch. of Ribeirao Preto - Univ. of Sao Paulo, Ribeirao Preto, Brazil

Abstract: Adolescent exposure to cannabinoids is proposed to be a risk factor for psychiatric conditions, particularly schizophrenia. Animal studies have indicated that a combination of repeated cannabinoid administration during adolescence with either neonatal prefrontocortical lesion, isolation rearing, or chronic NMDA receptor antagonism administration induces enhanced schizophrenia-like signs. The effects of adolescent exposure to CB1 receptor agonists, however, have not been tested in a developmental disruption model of schizophrenia. This was tested in the methylazoxymethanol (MAM) model, in which repeated treatment with the synthetic cannabinoid agonist WIN 55,212-2 (WIN; 1.2mg/kg) was extended over 25 days throughout puberty (postnatal days 40-65) in control and MAM rats. The rats received 20 injections, which were delivered irregularly to mimic the human condition. Adult rats were

tested for attentional set-shifting task and locomotor response to amphetamine, which was compared with *in vivo* recording from ventral tegmental area (VTA) dopamine (DA) neurons. As expected, MAM-treated rats showed impairment in the attentional set-shifting task, augmented locomotor response to amphetamine, and an increased number of spontaneously active DA neurons in the VTA. Adolescent WIN treatment in normal animals induced similar changes at adulthood as those observed in MAM-treated rats, supporting the notion that adolescence exposure to cannabinoids may represent a risk factor for developing schizophrenia-like signs at adulthood. However, contrary to our expectations, pubertal WIN administration did not exacerbate the changes in MAM-treated rats beyond that observed in WIN-treated normal rats. Indeed, WIN treatment actually attenuated the locomotor response to amphetamine in MAM rats without impacting DA neuron activity states. Altogether, our results indicate that the impact of cannabinoids during adolescence on schizophrenia models is more complex than may be predicted.

Disclosures: **F.V. Gomes:** None. **F.S. Guimaraes:** None. **A.A. Grace:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.05/E27

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

Title: Developmental antioxidant treatment in the neonatal ventral hippocampal lesion model of schizophrenia: effects on executive function and control

Authors: D. ACS, B. BENANZEA-FONTEM, D. LAFFERTY, *A. H. BRADY;
Psychology and Neurosci., St. Mary's Col. of Maryland, St Marys City, MD

Abstract: Animals with a neonatal ventral hippocampal lesion (NVHL), a developmental model of schizophrenia, exhibit impairments in executive function and control, including deficits in set-shifting and enhanced drug-seeking behavior. Attempts to reverse or prevent cognitive impairments in animal models have been largely unsuccessful, paralleling the low efficacy of antipsychotic treatments in alleviating cognitive symptoms in the clinic. Recent evidence supporting a role of oxidative stress in both schizophrenia and in the NVHL model led us to investigate the effects of developmental administration of the anti-oxidant N-acetylcysteine (NAC) on the adult emergence of behavioral impairments in the NVHL model. We examined two aspects of executive function and behavioral flexibility: set-shifting, previously demonstrated to be impaired in NVHL animals, and habit learning, not previously characterized

in the NVHL model. Regarding the latter, we hypothesized that NVHL rats would show a bias toward habit-based over goal-directed behavior, which may contribute to their enhanced motivation to self-administer drugs of abuse. Male Sprague-Dawley rats received bilateral hippocampal infusions of ibotenic acid (NVHL group) or artificial CSF (sham group) on postnatal day 7 (P7). Two groups of rats were given NAC treatment in the drinking water (900 mg/l) from P5 through P50 (juvenile administration). Another two groups were given the same NAC treatment from P35 to P50 (adolescent administration). At adulthood, all animals were trained to lever press, and then given an outcome devaluation procedure to assess sensitivity to changes in reinforcer value. A failure to decrease responding to the devalued reinforcer was characterized as a bias toward habit-based responding. Preliminary results suggest the presence of a modest bias toward habit-based responding in NVHL animals that was lessened by NAC administration. Animals were then trained on an operant set-shifting task where they were required to learn a position-based rule on the first day of testing (Set), and then shift to a visual cue-based rule on the second day of testing (Shift). As previously observed in this task, NVHL rats were unimpaired on learning the first rule (Set), but required more trials to reach criterion and made more errors than shams on the second rule (Shift). This set-shifting deficit was prevented by juvenile, but not adolescent, NAC administration. Together, these results replicate and extend the characterization of executive function impairments in the NVHL model, and suggest that antioxidant treatment is a promising direction in the treatment of cognitive symptoms of schizophrenia.

Disclosures: D. Acs: None. **B. Benanzea-Fontem:** None. **D. Lafferty:** None. **A.H. Brady:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.06/E28

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

Support: Oberlin College Grant-in-Aid

Title: Inhibition of luteinizing hormone rescues recognition memory and raises hippocampal GAD67 in an animal model of schizophrenia

Authors: *C. E. LYONS, A. W. SCHALER, A. J. RIORDAN, J. FRIED, T. A. PAINE, J. E. THORNTON;
Dept. of Neurosci., Oberlin Col., Oberlin, OH

Abstract: Schizophrenia is a psychiatric disorder characterized by debilitating and presently untreatable cognitive deficits. Some studies suggest that lowered levels of GAD67, the rate-limiting enzyme for GABA production, and reduced numbers of parvalbumin-expressing (PV+) GABA neurons in the cortex and hippocampus may underlie this cognitive loss. Concurrent research suggests that estradiol may help to alleviate cognitive symptoms in people with schizophrenia. Our lab recently showed that estradiol treatment reverses memory deficits in an animal model of schizophrenia. As one of estradiol's effects is reduction of luteinizing hormone (LH), we sought to determine whether inhibition of LH would also rescue cognition in this schizophrenia model. Furthermore we tested the hypothesis that estradiol and inhibition of LH act to restore memory by increasing PV+ neurons and/or GAD67 levels in the prelimbic cortex and/or hippocampus. Adult female rats were ovariectomized and implanted with either a blank or estradiol-filled silastic capsule. To induce schizophrenia-like symptoms, animals were injected with either PCP (phencyclidine, 2 mg/ml/kg) or vehicle bidaily for 7 days. Following a 7-day washout period, the LH-inhibiting drug Antide (a gonadotropin releasing hormone antagonist) or vehicle was administered 6 hours prior to cognitive testing on the Novel Object Recognition Task. For both the prelimbic cortex and the hippocampus, the numbers of parvalbumin immunopositive (PV+) cells were determined using immunohistochemistry and GAD 67 levels were measured via Western blots. As previously seen, estradiol reversed object recognition deficits induced by PCP. Furthermore, Antide also rescued memory deficits and increased GAD67 levels in the dorsal hippocampus of PCP-treated animals. PCP did not significantly alter the number of PV+ cells in either the prelimbic cortex or hippocampus (CA1, CA3, or dentate gyrus). These results suggest that inhibition of luteinizing hormone rescues cognition by restoring GAD67 in a PCP-model of schizophrenia. Hence, lowered levels of hippocampal GAD67 may be responsible for memory deficits in schizophrenia, and hormonal manipulations may offer promising treatments for the devastating cognitive symptoms of this disorder.

Disclosures: C.E. Lyons: None. **A.W. Schaler:** None. **A.J. Riordan:** None. **J. Fried:** None. **T.A. Paine:** None. **J.E. Thornton:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.07/E29

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

Support: Swiss National Science Foundation Nr. 310030_146217 (UM)

European Molecular Biology Organization Short-Term Fellowship (MAL)

Title: GABAergic promoter remodeling in an immune-mediated neurodevelopmental mouse model with relevance to schizophrenia

Authors: *M. A. LABOUESSE[1], E. DONG[2], W. LANGHANS[1], D. GRAYSON[2], A. GUIDOTTI[2], U. MEYER[1,3];
[1]ETH Zurich, Schwerzenbach, Switzerland; [2]The Psychiatric Inst., Univ. of Illinois at Chicago, Chicago, IL; [3]Univ. of Zurich-Vetsuisse, Zurich, Switzerland

Abstract: Accumulating evidence has identified epigenetic abnormalities in post-mortem brains of schizophrenic patients, including aberrant levels of methylating/demethylating enzymes and enhanced promoter methylation of disease-relevant genes such as glutamic acid decarboxylase 1 (GAD1), the rate-limiting enzyme for gamma-aminobutyrate (GABA) biosynthesis. These findings have led to the hypothesis that schizophrenia pathology may (at least partly) emerge from a dysregulation of epigenetic networks. However, the precise environmental and genetic factors triggering such epigenetic modifications remain largely elusive. Maternal infection is a recognized environmental risk factor for neurodevelopmental psychiatric diseases such as schizophrenia. Experimental rodent models support this idea by demonstrating disease-relevant long-term behavioral abnormalities following maternal immune activation that associate with reductions in cortical GAD1 and GAD2 levels. In the present study, we thus aimed at identifying the presence of aberrant epigenetic marks at GAD1 and GAD2 promoter regions and their potential association with cognitive deficits in offspring born to immune-challenged mothers. We used a well-established mouse model of maternal exposure to the viral mimic polyriboinosinic-polyribocytidilic acid (PolyI:C). Methylated-and hydroxymethylated DNA immunoprecipitation techniques revealed enhanced methylation and hydroxymethylation levels at GAD1 and GAD2 promoters in offspring born to PolyI:C-treated mothers. These effects were specific to the promoter region and associated with a concomitant reduction in GAD1 and GAD2 mRNA levels. Hypermethylation of GAD1 and GAD2 promoters was accompanied by enhanced chromatin binding of methyl CpG binding protein 2 (Mecp2). Further behavioral analyses revealed marked deficits in social interaction and spatial working memory in PolyI:C offspring. Interestingly GAD1 (but not GAD2) promoter methylation and hydroxymethylation levels negatively correlated with spatial working memory performance and social interaction scores, supporting a functional role for these epigenetic modifications in the emergence of behavioral deficits induced by maternal immune activation. Though the origins of GABAergic abnormalities in schizophrenia are likely multifactorial, the present findings delineate one putative mechanism by which maternal infection, a risk factor for the disease, may affect the integrity of GABAergic systems in affected patients. These chromatin-remodeling events may in turn possibly contribute to the emergence of behavioral symptoms that characterize schizophrenia.

Disclosures: **M.A. Labouesse:** None. **E. Dong:** None. **W. Langhans:** None. **D. Grayson:** None. **A. Guidotti:** None. **U. Meyer:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.08/E30

Topic: A.09. Adolescent Development

Support: NHMRC Project Grant APP1044887

Title: Interactions between testosterone and brain-derived neurotrophic factor on brain development - with relevance to schizophrenia

Authors: *X. DU[1], M. VAN DEN BUUSE[2], R. A. HILL[1];
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Abstract: Reduced expression of the key neurotrophin brain-derived neurotrophic factor (BDNF) is a common biological feature in schizophrenia patients. Restoration of its levels is a target of therapeutics. Our group has previously shown in mice that forebrain BDNF levels correlate with pubertal elevations in testosterone (TT), suggesting TT is able to drive BDNF expression during adolescence. Men are more likely to develop schizophrenia than women and male patients suffer worse outcomes. Hence, the relationship between TT and BDNF during adolescence, the period of dynamic brain system alterations and the phase leading up to the window of peak onset for schizophrenia, becomes immensely important with regards to explaining sex differences and disease mechanisms of schizophrenia. Utilising both wild-type (WT) and BDNF heterozygous (het) mice, we found that reduced BDNF alters the way forebrain GABAergic interneurons develop throughout adolescence, suggesting interplay between TT and BDNF. We further examined the effect of pre-pubertal hormone manipulation on drug-models of schizophrenia. Prepubescent mice were gonadectomised and implanted with placebo, TT or dihydrotestosterone (DHT). Upon adulthood, amphetamine and MK801 induced hyperactivity and pre-pulse inhibition (PPI) were tested to examine the regulation on the dopamine and NMDA systems respectively. Our data show an intricate interaction between TT and BDNF, with TT but not DHT able to mediate MK801 and amphetamine-induced hyperactivity in WT mice, signifying the importance of the conversion of TT to estrogen in mediating this effect. In het mice however, while TT was able to suppress amphetamine-induced hyperactivity, only DHT was able to suppress MK801-induced hyperactivity. This indicates altered reliance in the het mice towards androgen receptor signalling in mediating NMDA pathways. In the WT mice, TT but not DHT was able to increase PPI after amphetamine, again signifying conversion to estrogen to be involved. Het mice show an overall enhanced startle response but no changes in

PPI in response to hormone manipulations. Further work is being carried out using cell targeted viral gene knockdown to deactivate BDNF and sex hormone signalling in specific areas of the brain during prepubescence to allow more targeted and higher resolution analyses of the effects of the interaction between sex hormone and BDNF. The multifaceted relationships between sex hormone signalling and BDNF may provide novel and effective targets for pharmacological treatments. Our results highlight the possibility of using various agents to increase BDNF and/or sex hormone signalling to mitigate symptoms of schizophrenia.

Disclosures: **X. Du:** None. **M. Van den Buuse:** None. **R.A. Hill:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.09/E31

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

Support: NIH Grant R01MH085666

Title: Accelerated GSK3β activity promotes spine elimination by facilitating LTD in developing prefrontal cortex in a schizophrenia model

Authors: *B. XING, W.-J. GAO;
Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Schizophrenia is a neurodevelopmental disorder with its peak onset during adolescence. Although the pathophysiology of schizophrenia remains largely unknown, synaptic dysfunction, especially dendritic spine loss in the prefrontal cortex (PFC), appears to be a consistent hallmark trait associated with this disorder. A fundamental question that haunts the field is when and how this spine loss happens during early development in schizophrenia. Here we address this question using a neurodevelopmental model of gestational exposure to a neurotoxin methylazoxymethanol (MAM). We found that MAM exposure induced a significant decrease in inhibitory serine 9 phosphorylation of GSK3β during the juvenile period of development, indicating an age-dependent increase in GSK3β activity. This transient, accelerated GSK3β activity dramatically facilitated long-term depression (LTD) by decreasing its threshold of induction in juvenile PFC neurons, which can be efficiently blocked by GSK3β inhibitors. The facilitated induction of LTD in MAM-exposed rat PFC only exists within a brief window of juvenile development, but it obviously prolongs the critical period for synaptic elimination. Indeed, MAM exposure resulted in a significant decrease in spine number in apical, but not

basal, dendrites of both layer II/III and layer V pyramidal neurons; and more importantly, treatment with a GSK3β inhibitor in juvenile development effectively rescued dendritic spine deficits in adult rats. These results highlight the importance of the developmental trajectory of GSK3β for synaptic plasticity and spine maturation during a critical period of PFC development, suggesting that alterations in synaptic plasticity related to GSK3β dysregulation in juveniles may contribute to the spine loss this is a classical feature of the pathological processes of schizophrenia. *Supported by NIH R01MH085666 to W.J. Gao.*

Disclosures: B. Xing: None. **W. Gao:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.10/E32

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

Support: NIH DA024746

Tourette Syndrome Association TSA-50841

Institute of International Education IIE-SRF fellowship

Title: A methionine induced prenatal animal model of schizophrenia

Authors: *L. WANG, A. ALACHKAR, S. LEE, Z. WANG, G. ABBOTT, O. CIVELLI; Pharmacol., Univ. of California, Irvine, Irvine, CA

Abstract: Methionine directs methylation which is central to epigenesis and is recognized as an important factor in the etiology of schizophrenia. Methionine administration to patients with schizophrenia has been reported to exacerbate the psychotic symptoms. Further, our previous study showed that repeatedly administration of L-methionine to adult mice led to behavioral changes that reflect the three types of schizophrenia-like symptoms. Here we established a developmental mouse model of schizophrenia. Pregnant mice were repeatedly administered L-methionine during the third trimester. We report that the male offspring of the female treated with L-methionine exhibited behavioral changes that reflect the three types of schizophrenia-like symptoms since adolescent hood until adulthood (positive, negative or cognitive deficits). We also show that the schizophrenia-like responses induced by the methionine treatment are reversed differentially by haloperidol and clozapine. Moreover, we show that these male offspring exhibited changes in brain size, neuronal development and genes associated with schizophrenia.

Our model relies on an essential natural amino acid and on an environmental and developmental intervention and may offer an additional tool for assessing novel antipsychotics.

Disclosures: **L. Wang:** None. **A. Alachkar:** None. **S. Lee:** None. **Z. Wang:** None. **G. Abbott:** None. **O. Civelli:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.11/E33

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

Support: NIH DA024746

TSA-50841

Institute of International Education IIE-SRF fellowship

Title: Role of Prenatal methionine load in Schizophrenia

Authors: ***A. ALACHKAR**, L. WANG, S. LEE, Z. WANG, G. ABBOTT, O. CIVELLI; Pharmacol., Univ. of California Irvine, Irvine, CA

Abstract: Exposure to environmental factors during gestation results in changes in the fetal epigenome. DNA methylation is a critical epigenetic factor that modifies expression of genes involved in vital neural functions such as neurogenesis and differentiation. Maternal methyl-donor diet at the conception influences the DNA methylation and leads to permanent changes in the offspring gene. Abnormal DNA methylation has been implicated in schizophrenia. Here we test the effect of methionine overload during the third trimester of gestation on the neurodevelopment and behavioral responses of the male offspring in mice. Pregnant mice were repeatedly administered L-methionine for the last 7 days of pregnancy. Adults offspring were tested in a battery of behavioral assays. Microarray of mRNA was performed to determine changes in gene expression. Our data show that prenatal methionine administration in the period E14-E20 of the gestation produced permanent changes in the adult pups genes that are involved in neurodevelopment including Npas4, ARC, Fos, and FGF1. These changes were accompanied with the changes in the behavioral phenotype that mimic schizophrenia symptoms including positive, negative symptoms and cognitive deficits.

Disclosures: **A. Alachkar:** None. **L. Wang:** None. **S. Lee:** None. **Z. Wang:** None. **G. Abbott:** None. **O. Civelli:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.12/E34

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

Support: NIH Grant MH57440

Title: Altered context processing in the MAM rat model of schizophrenia: relationship to dopamine dysfunction

Authors: *K. M. GILL, A. GRACE;
Dept. of Neurosci., Univ. Pittsburgh, Pittsburgh, PA

Abstract: The appropriate acquisition of context information is vital for the disambiguation of neutral and aversive contexts, a process known to be impaired in schizophrenia (SZ) patients. In normal rats, repeated pre-exposure to the same context results in a transition from facilitation to a suppression of context-induced fear responses. This pattern of progressive alterations in the response to context information relies on intact amygdala and hippocampal activity. There is evidence for altered activity within amygdala and ventral hippocampus in SZ, which could underlie impaired context processing. In the present study, we examined contextual fear learning in the methylazoxymethanol acetate (MAM) developmental model of SZ in the rodent. Specifically, we tested the time course of context pre-exposure effects on fear learning in MAM animals in the immediate shock fear learning paradigm. Given the known dopamine (DA) system pathology in MAM animals, amphetamine-induced locomotion was used as a means of assessing DA system activation and its relationship to prior contextual fear learning. Saline and MAM-treated offspring were trained in a modified context pre-exposure fear conditioning paradigm involving varying amounts of context pre-exposure (0,1,3,5,7, and 10 days). The last day of context pre-exposure took place 24 hours prior to conditioning. For conditioning, all rats received a 0.4-mA, 2-sec shock 120-sec after being placed in the conditioning context. 24-hr after conditioning, animals were placed in the same context, and freezing scored for 5-min. At least one week following fear conditioning, locomotor activity was assessed following an acute injection of amphetamine (0.5 mg/kg, i.p.). Saline rats demonstrated the expected increase in freezing behavior following a single pre-exposure session. In contrast, MAM animals exhibited reduced fear responses compared to normal rats. In addition, MAM animals failed to show the

expected context-induced facilitation of fear responses. Surprisingly, MAM rats demonstrated a blunted amphetamine-induced locomotor response compared to saline animals following fear exposure. In addition, the enhanced locomotor response in saline animals was context-dependent and greatest at fewer pre-exposure sessions. MAM rats were unable to engage consolidated contextual information even with extensive context pre-exposure occurring prior to fear conditioning. In addition, fear learning produced a persistent reduction in DA system activation in MAM rats. These data provides insight into the behavioral pathology regarding how disruption of hippocampal-dopamine interactions in SZ may impact context processing.

Disclosures: **K.M. Gill:** None. **A. Grace:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.13/E35

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

Support: NIH Grant MH57440

Title: Heightened amygdala theta response oscillation in rats with MAM model of schizophrenia

Authors: *Y. DU[1], A. A. GRACE[2];
[1]Dept. of Neurosci., [2]Neuroscience, Psychiatry and Psychology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Previous studies from our group have shown that rats exposed during gestational day 17 to the mitotoxin methyl azoxymethanol acetate (MAM) exhibit behavioral, pharmacological, and anatomical characteristics consistent with an animal model of schizophrenia. Recently, we have observed abnormal stress response, and also a higher level of anxiety in both adolescent and adult MAM-treat rats, which is consistent with altered responses to stress and emotional stimuli in schizophrenia patients. Moreover, heightened stress sensitivity and level of anxiety are observed in children and adolescents at a risk of developing schizophrenia later in their life. Studies have observed abnormal activity of the amygdala in schizophrenia patients, particularly during tasks with emotional stimuli. The amygdala sends extensive projections to the PFC and hippocampus; regions commonly reported to be altered in postmortem and structural imaging studies of schizophrenia. In addition, pharmacological activation of the amygdala can mimic the alterations observed in the schizophrenia brain, such as loss of parvalbumin interneurons in the hippocampus. The amygdala is a central brain region in stress response and emotional regulation.

Thus abnormal activity of the amygdala could contribute to stress hypersensitivity in MAM-treated rats. In this study, stainless steel wire electrodes were implanted into the basolateral amygdala (BLA)/ lateral amygdala (LA). After one week recovery, rats were trained on a standard fear conditioning paradigm in which a 2-s tone presentation was paired with a mild footshock (0.45mA) for 10 times, with a pseudorandom inter-trial interval (45-100 s). Rats were returned to a different chamber 24 hr later, and local field potentials (LFPs) were recorded. Tone-evoked change in LFP power was calculated by normalizing LFP power in the 10-s period after tone presentation to that in the 10-s period before tone presentation. Preliminary results showed an increase after tone presentation in theta band (4-8 Hz) in both Sal and MAM rats. The overall increase in the theta band response was not significantly different when comparing Sal and MAM rats (MAM rats showed a trend of larger increase, $p=0.1$). However, the MAM rats showed a marked peak of increase in oscillatory activity around 5.5 Hz that was significantly larger ($p<0.01$, t-test) (160%+/-30%, n=3) than that in Sal rats (80%+/-20%, n=5). This highly synchronized activity in the amygdala of MAM rats may be related to the hypersensitivity to stress in these rats.

Disclosures: Y. Du: None. **A.A. Grace:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.14/E36

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

Support: CIHR; MOP 246144

NSERC

Title: Adolescent cannabinoid exposure leads to molecular and neuronal alterations in the mesocorticolimbic system and behavioural impairments resembling schizophrenia symptomology

Authors: *J. RENARD, M. LOUREIRO, L. G. ROSEN, W. J. RUSHLOW, S. R. LAVIOLETTE;
Anat. and Cell Biol., Western Ontario Univ., London, ON, Canada

Abstract: Marijuana (MJ) is the most widely used illicit drug among adolescents. Considerable clinical evidence now supports the hypothesis that adolescent exposure to high levels of delta-9-

tetrahydrocanabinol (THC), the principal psychoactive component in MJ, increases the risk of developing neuropsychiatric symptoms in early adulthood, characterized by emotional dysregulation and affective disturbances. This MJ-associated risk is believed to be related to increasing relative levels of THC found within commonly used cannabis strains. Importantly, cannabinoids strongly interact with the mesocorticolimbic dopamine (DA) pathway, a neural system that is believed to underlie the neuropsychopathological effects of schizophrenia. Given the exponential rise in cannabis use for both recreational and therapeutic purposes, an important question concerns the differential risk profile between adolescent, vs. adulthood THC exposure. In the present study, we used an integrative combination of molecular analyses, behavioural studies and *in vivo* neuronal electrophysiology to compare the effects of adolescent vs. adulthood exposure to THC in rats. Our results reveal important distinctions between the effects of adolescent vs. adulthood THC exposure on various schizophrenia-related behavioural, molecular and neuronal phenotypes: 1) Adolescent, but not adult exposure, causes profound psychotomimetic symptoms including social cognition, motivational, sensorimotor gating and depressive-like behavioural disturbances, 2) Adolescent, but not adult exposure to THC profoundly decreases prefrontal cortical (PFC) phosphorylated protein levels of GSK-3 alpha and beta isoforms, mTOR and p70S6 kinase, similar to findings observed in post-mortem PFC analyses from schizophrenia and mood disordered patients, 3) Adolescent, but not adult exposure, leads to a profound state of hyper-dopaminergia, evidenced by increased levels of spontaneous firing frequency and bursting rates in DA neurons of the ventral tegmental area (VTA), an effect that is mechanistically consistent with our observed molecular alterations showing decreased PFC expression of phosphorylated GSK-3. Our findings represent the first demonstration that adolescent THC exposure may simultaneously lead to neuronal, behavioural and molecular phenotypes closely resembling those observed in schizophrenia. In addition, our findings have profoundly important implications for public health policy development related to regulating adolescent vs. adulthood exposure to high-THC strains of cannabis.

Disclosures: **J. Renard:** None. **M. Loureiro:** None. **L.G. Rosen:** None. **W.J. Rushlow:** None. **S.R. Laviolette:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.15/E37

Topic: A.09. Adolescent Development

Support: NIMH R01-MH086507

Title: Downregulation of parvalbumin in the prefrontal cortex during adolescence causes enduring disruptions of prefrontal processing of ventral hippocampal inputs

Authors: *A. CABALLERO, D. R. THOMASES, E. FLORES-BARRERA, K. Y. TSENG; Cell. and Mol. Pharmacol., Rosalind Franklin Univ., North Chicago, IL

Abstract: One consistent observation during the development of cortical structures is the activity-dependent upregulation of the calcium binding protein parvalbumin (PV) in local GABAergic interneurons. In the prefrontal cortex (PFC) of mammals, this finding is recapitulated during postnatal development with a significant increase in PV expression during adolescence which reaches relatively stable levels once in adulthood. Given the role of PV in the regulation of neurotransmitter release, we asked whether the increase in PV during adolescence was necessary to sustain PFC GABAergic function. For this purpose, we utilized an RNAi strategy to directly downregulate prefrontal PV levels during adolescence and tested GABAergic function during adulthood. Our results indicate that a decrease in PV expression (~30%) in the PFC was sufficient to decrease the frequency of IPSC onto pyramidal neurons to levels seen in juvenile/early adolescent animals. Moreover, these animals failed to properly process afferent information in the PFC, as measured by a suppression of the normal inhibitory LFP resulting from ventral hippocampal stimulation at 20 and 40 Hz. These electrophysiological findings were mirrored by an impaired extinction response in a trace fear conditioning assay, a behavioral paradigm that requires intact PFC-ventral hippocampus connectivity. We therefore conclude that the developmental upregulation of PV in the PFC is required for proper GABAergic transmission and local inhibitory control of afferent drive, and any insults that prevent it will lead to a disinhibited PFC. Importantly, these results suggest there is a critical window of plasticity during which PV upregulation supports the acquisition of mature GABAergic phenotype necessary to sustain adult PFC functions.

Disclosures: **A. Caballero:** None. **D.R. Thomases:** None. **E. Flores-Barrera:** None. **K.Y. Tseng:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.16/E38

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

Support: NIH Grant MH091407

Veteran's Affairs VISN 22 MIRECC

Title: Aspirin treatment prevents behavioral deficits in a maternal immune activation model in mice

Authors: *A. KHAN[1], J. LUCERO[2], M. BEHRENS[2], M. A. GEYER[1,3], S. B. POWELL[1,3]; [1]Dept. of Psychiatry, Univ. of California San Diego, LA Jolla, CA; [2]Salk Inst. for Biol. Studies, La Jolla, CA; [3]Res. Service, VA San Diego Healthcare Syst., San Diego, CA

Abstract: Activated inflammatory responses in brain and periphery have been implicated in a variety of neuropsychiatric and neurodevelopmental disorders, including schizophrenia and autism. These inflammatory responses are likely triggered by various genetic and environmental factors. For example, epidemiological studies indicate that the risk of developing schizophrenia and autism is increased by prenatal exposure to bacterial or viral infection during pregnancy. There is also evidence for an ongoing neuroinflammation in schizophrenia patients, including elevations in pro-inflammatory cytokines (e.g. IL-6, IL-12, IL-1β, TNF-α, interferon-γ) and activated microglia. Thus, because there is evidence that neuroinflammation occurs in acutely ill first-episode patients, anti-inflammatory drugs may be an effective treatment. Microglia are the first line of defense in brain and have recently become the focus of possible therapeutic targets in schizophrenia. Here we investigated the effects of the non-steroidal anti-inflammatory drug (NSAID), acetylsalicylic acid (aspirin), in the maternal immune activation (MIA) model in mice. We hypothesize that administration of aspirin will prevent the behavioral deficits by possibly blocking the microglial activation in brain. To produce MIA polyriboinosinic-polyribocytidilic acid (Poly I:C) was injected to pregnant C57BL/6J dams at two gestational time points (3 mg/kg on day 12.5 and 1.5 mg/kg on 17.5) according to our previously published methods. Pups born to poly I:C- or vehicle-exposed dams (control group) were assigned to either water or aspirin treatment groups. Aspirin was administered in water (480μg/mouse/day) from postnatal day 30-72. Mice were subsequently tested for schizophrenia-like behavioral phenotypes and brains were taken for assessment of microglia. MIA mice showed deficits in spatial working memory as measured in the t-maze spontaneous alternation task. Aspirin administration prevented the spatial working memory deficit in MIA mice [Prenatal exposure x postnatal treatment interaction, $[F(1,31)=5.96, p<0.05]$. We are currently examining the number and shape of microglia in frontal cortex and hippocampus. Our findings will help determine whether anti-inflammatory drugs may target microglia activation and show promise as a treatment for disorders that involve ongoing inflammation such as schizophrenia and autism.

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Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.17/E39

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

Support: NIMH Grant MH103222-01

Title: Prenatal dynamics of kynurenine pathway metabolism in rodents

Authors: *N. GOEDEN[1], A. POCIVAVSEK[2], F. M. NOTARANGELO[2], S. BEGGIATO[2], A. BONNIN[3], R. SCHWARCZ[2];
[1]USC, Los Angeles, CA; [2]Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Cell and Neurobio., Keck Sch. of Med. of the Univ. of Southern California, Los Angeles, CA

Abstract: Malfunction of the kynurenine pathway (KP) of tryptophan degradation has been linked to the pathophysiology of schizophrenia (SZ). Specifically, elevated levels of the KP metabolite kynurenic acid (KYNA), which are found in postmortem brain and cerebrospinal fluid of individuals with SZ, may be causally related to cognitive deficits seen in the disorder. Moreover, in line with the neurodevelopmental hypothesis of SZ etiology, experimental increases of KYNA in the fetal brain cause delayed up-regulation of brain KYNA and cognitive impairments in adulthood (Pocivavsek et al. 2014). To provide insights into the prenatal dynamics of KP metabolism in this context, we now used L-kynurenine ("kynurenine"), the immediate bioprecursor of KYNA, as an experimental tool. Using *ex vivo* technology (Goeden et al. 2012), we perfused increasing concentrations of kynurenine (0.5 to 100 μM) for a total of 100 min through intact placentas harvested from pregnant mice on embryonic day (ED) 18, and determined kynurenine and KYNA levels in 20-min perfusate fractions obtained from the umbilical vein. This experiment revealed a significant transplacental transfer of kynurenine, and suggests that, if any, only a small fraction of maternal kynurenine is converted to KYNA in the placenta under physiological conditions. Interestingly, transplacental transfer rates decreased when 100 μM kynurenine was used, indicating that maternal-fetal transport of kynurenine is limited and saturable, and therefore likely involves a specific transporter. To further study the impact of elevated maternal kynurenine on fetal KP metabolites, we then used an *in vivo* approach and injected pregnant rats with kynurenine (100 mg/kg, i.p.) on ED18. Animals were sacrificed, and tissues were collected 90 min later. Analysis of KP metabolites revealed significant increases in kynurenine, KYNA and 3-hydroxykynurenine (3-HK) levels in both the maternal and the fetal brain. Taken together, these *ex vivo* and *in vivo* approaches begin to define maternal and placental roles in determining the characteristics of the KP in the fetal brain.

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Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.18/E40

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

Support: NIH R15DA034912-01A1

Title: A test of the aversive versus the rewarding effects of nicotine in rats neonatally treated with quinpirole: Analysis of brain plasticity mechanisms

Authors: S. L. KIRBY[1], E. D. CUMMINS[1], D. J. PETERSON[1], A. R. DENTON[1], *J. M. DOSE[2], R. W. BROWN[1];
[1]Psychology, East TN State Univ., Johnson City, TN; [2]Psychology, St. Norbert Col., De Pere, WI

Abstract: Neonatal quinpirole (a dopamine D2-like receptor agonist) treatment to rats has been shown to increase dopamine D2 receptor sensitivity throughout the animal's lifetime, and increased dopamine D2 sensitivity is a hallmark of schizophrenia. Schizophrenics are three to four times more likely to smoke than the normal population, but there is no delineating mechanism. The objective of this study was to behaviorally test a rewarding versus aversive dose of nicotine in adolescent rats neonatally treated with quinpirole tested in a conditioned place preference (CPP) paradigm. Brain tissue was analyzed for several proteins known to be important in brain plasticity. Rats were neonatally treated with quinpirole from postnatal days (P)1-21 and raised to P40 without any further drug treatment. After two drug free preference tests were administered in a three-chambered place preference shuttle box at P41-42, animals were conditioned with saline, a 0.6 or a 1.8 mg/kg free base dose of nicotine for eight consecutive days from P43-50. A drug free post-conditioning test was given 24 h after conditioning on P51. Time spent in the paired and unpaired context were measured on the post-conditioning test which was subtracted from time spent in the same contexts on the pre-conditioning test and used as the behavioral dependent measure. Results revealed that neonatal quinpirole enhanced the rewarding associative effects of the lower dose of nicotine (0.6 mg/kg free base) compared to animals neonatally treated with saline and conditioned with the same dose of nicotine, which showed a slight place preference compared to saline controls. Interestingly, although neonatal saline animals conditioned with the higher dose of nicotine

demonstrated conditioned place aversion, neonatal quinpirole treated animals demonstrated no aversion to this same dose. Therefore, rats neonatally treated with quinpirole demonstrate an enhancement to the rewarding properties of nicotine, but do not demonstrate an aversion to higher doses of nicotine. Brain tissue was removed on P52 and is currently being analyzed for the phosphorylated protein mammalian target of rapamycin (mTOR), ribosomal protein S6, and cyclic AMP response element binding protein (pCREB). All of these proteins have been implicated in the regulation of synaptic growth and synaptic strength. The behavioral data are congruent with recent self-administration data in our lab, and suggest that increases of dopamine D2 sensitivity may blunt aversive aspects of nicotine.

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Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.19/E41

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

Support: NIH P50 MH103222

Title: Maternal, placental and fetal KYNA production in mouse tissue slices

Authors: *S. BEGGIATO, F. M. NOTARANGELO, R. SCHWARCZ;
Psychiatry, Maryland Psychiatric Research, Univ. of Maryl, Baltimore, MD

Abstract: Studies in animals and humans suggest a pathophysiologically significant association between elevated brain kynurenic acid (KYNA) levels on one hand and major brain diseases such as schizophrenia on the other (Nat. Rev. Neurosci., 13: 465-477, 2012). Prenatal exposure to kynurenine, the direct bioprecursor of KYNA, induces cognitive impairments reminiscent of SZ in adult rats (Neuropharmacology, 90: 33-41, 2015), suggesting a developmental dimension of the link between KYNA and SZ. Pharmacological agents that mitigate the elevations in brain KYNA levels, for example inhibitors of KYNA's biosynthetic enzyme kynurenine aminotransferase II (KAT II; Schiz. Bull., 40: S152-S158, 2014), might be useful to attenuate or reverse the detrimental effects and possibly guide the development of fundamentally new clinical interventions. In this context, the present *in vitro* study was designed to evaluate the ability of various tissues, obtained from pregnant FVB/N mice on embryonic day 18, to synthesize KYNA from its immediate bioprecursor L-kynurenine (L-KYN). Specifically, we used freshly prepared

tissue slices from maternal brain, placenta and fetal brain, to investigate de novo KYNA synthesis under physiological conditions (incubation in Ringer buffer) or using L-KYN (10 μM) to drive KYNA formation. Tissues were incubated for 1 hour at 37º C, and KYNA levels were determined in the incubation medium. Basal KYNA levels measured after incubation of fetal brain and placenta slices were 4-5 times higher than after incubation of maternal brain slices. Incubation with L-KYN significantly increased KYNA levels in all tissues. Incubation in the presence of aminooxyacetic acid (1 mM), a non-specific KAT inhibitor, completely prevented L-KYN-induced KYNA formation in all tissues. More selective KAT inhibitors (1 mM glutamine to block KAT I, 1 mM BFF-122 to inhibit KAT II, and 500 μM aspartate to inhibit KAT IV; J. Neurochem., 102: 103-111, 2007) were used to further characterize the roles of the various KYNA-synthesizing enzymes in the three tissues examined. This approach increases the understanding of the dynamics of kynurenine pathway metabolism during pregnancy in basal conditions as well as after exposure to L-KYN. Exploring the source and the regulation of KYNA production in the fetus could be particularly important for developing therapeutic interventions in disorders that have etiological links to events in early life.

Disclosures: **S. Beggiato:** None. **F.M. Notarangelo:** None. **R. Schwarcz:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.20/E42

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

Support: NIH R15DA034912-01A1

Title: Neonatal quinpirole treatment enhances nicotine self-administration in adult rats

Authors: *R. W. BROWN, L. A. BEUTTEL, E. M. ODINEAL, E. D. CUMMINS, G. KASSEM, C. A. BRADLEY, M. I. PALMATIER;
East TN State Univ., Johnson City, TN

Abstract: This study analyzed nicotine self-administration in adult male rats neonatally treated with the dopamine D2/D3 agonist quinpirole. Our laboratory has shown that neonatal quinpirole treatment produces increases in sensitivity of dopamine D2-like receptors throughout the animal's lifetime without a change in receptor number, consistent with schizophrenia. Schizophrenics are 3 to 4-fold more likely to smoke cigarettes than the general population, but no mechanism for this comorbidity has been delineated. Male Sprague-Dawley rats were given

single intraperitoneal (i.p.) injections of either quinpirole (1mg/kg) or saline from postnatal days (P)1-21. Animals were then socially housed and raised to adulthood (P60). Rats were reduced to 85% of their free-feeding body weight and shaped to press a nose poke for a sucrose reward. The schedule of reinforcement during 15-min sessions was increased from an FR1 to FR5 schedule of reinforcement as animals acquired nose poke for a reward. After acquisition, rats were surgically implanted with an indwelling jugular catheter before nicotine self-administration commenced. Responding on an active nose poke was reinforced with nicotine delivered in a volume of 0.1 ml/kg, whereas responding on an inactive lever had no consequence. Once animals demonstrated stable responding on the FR schedule, animals were given the opportunity to respond on a progressive ratio (PR) schedule. Results showed that animals neonatally treated with quinpirole demonstrated an increase in the motivation to self-administer nicotine compared to controls administered neonatal saline at the highest dose tested (0.9 mg/kg). Neonatal quinpriole animals demonstrated a 4-fold increase in the number of active lever presses and the number of reinforcers obtained at this dose as compared to neonatal saline controls. These results show that increases in dopamine D2 sensitivity increases the motivation to self-administer nicotine, and provides further validation of the neonatal quinpirole treatment as a model of schizophrenia. Future work will analyze higher doses, such as 1.2 mg/kg to investigate whether neonatal quinpirole results in an increased motivation to self-administer doses found to be aversive in controls.

Disclosures: R.W. Brown: None. **L.A. Beuttel:** None. **E.M. Odineal:** None. **E.D. Cummins:** None. **G. Kassem:** None. **C.A. Bradley:** None. **M.I. Palmatier:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.21/E43

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

Support: PAPIIT IN302512-3

PAPIME PE300715

Title: Evaluation of chronic administration of nicotine in the NVHL model of schizophrenia

Authors: *A. OSTOS VALVERDE[1,2], D. B. PAZ-TREJO[1,3], A. MARTÍNEZ-TORRES[2], H. SANCHEZ-CASTILLO[1,3];
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Inst. de Neurobiologia, UNAM, Juriquilla, Queretaro, Mexico; [3]Sociedad Iberoamericana de Neurociencia Aplicada, Mexico City, Mexico

Abstract: Schizophrenia is a neuropsychiatric disorder characterized by three groups of symptoms: positive, negative and cognitive. Antipsychotic has been the primary treatment. It has been widely report that schizophrenic patients consume tobacco in a high rate. This gives place to the self-medication hypothesis, which postulates that patients consume nicotine in an attempt to ameliorate the cognitive deficits untreated by the classic medication. The goal of the work was to evaluate the effects of chronic administration of nicotine in the Neonatal Ventral Hippocampal Lesion (NVHL) model of schizophrenia. Subjects were 68 male Sprague-Dawley rats, which were trained in a radial labyrinth. After 8 sessions of training, delays in the execution were introduced (10, 30, 90 or 180 seconds). In this phase, rats were able to see the context of each arm. The last evaluation was made with an opaque confinement of 180 seconds. Chronic administration of nicotine was given with a subcutaneous injection of 0.5 mg/kg twice a day. The morphology of granular neurons of the hippocampus and pyramidal neurons of the cortex were evaluated using rapid Golgi. The obtained results indicate that the NVHL rats commit more errors and spend more time re-entering arms that no longer content the reinforcer. The deficits in execution were more marked in the performance with delays, where the 10 and 30 seconds interruption significantly impair the execution of the NVHL rats. The chronic administration of nicotine improved the execution of the NVHL animals diminishing the number of errors and time spent in committing errors. The results of the present work support the proposal of the self-medication hypothesis in schizophrenia, since the chronic administration of nicotine was effective in ameliorating the cognitive deficits in working memory shown by the NVHL animals. It can be conclude that the nicotine display a pro-cognitive effect because it was administered in a chronic fashion, versus acute administration shown in other reports. It has been previously reported that NVHL rats display deficits in the excitatory-inhibitory regulation of the cortex, that relies in the pyramidal neurons of this area, and that this deficit is closely related to the cognitive impairments of the rats. Because of this, it can be discussed that the pro-cognitive effect of the nicotine is mediated by the effect of this drug in the pyramidal neurons of the cortex, normalizing its morphology and function.

Disclosures: **A. Ostos Valverde:** None. **D.B. Paz-Trejo:** None. **A. Martínez-Torres:** None. **H. Sanchez-Castillo:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.22/E44

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

Title: Prolonged impact of perinatal exposure to phencyclidine on brain arginine metabolism in rats

Authors: *Y. R. JING, L. T. KNOX, H. ZHANG, P. LIU;
Anat., Univ. of Otago, Dunedin, New Zealand

Abstract: Schizophrenia is a debilitating psychiatric disorder associated with prominent prefrontal and hippocampal dysfunction. Neurodevelopmental disruption and N-methyl-D-aspartate (NMDA) glutamate receptor hypofunction have been linked to the aetiology and/or pathophysiology of the disease. Phencyclidine (PCP), a non-competitive NMDA receptor antagonist, induces schizophrenia-like symptoms and cognitive decline in health individuals. Recent genetic linkage and post mortem studies suggest that altered metabolism of L-arginine, a semi-essential amino acid with a number of bioactive metabolites, may also contribute to the pathogenesis of schizophrenia. The present study aimed to investigate how perinatal treatment with PCP affected brain arginine metabolism in rats during the perinatal period and adulthood. Male Sprague-Dawley pups received PCP (10 mg/kg) or saline (5 ml/kg) subcutaneously on postnatal days (PN) 7, 9 and 11. Animals were sacrificed on PN 13 (n = 6 per group) or PN 100 (n = 8 per group), and the prefrontal cortex (PFC) and hippocampus (the CA1, CA2/3 and dentate gyrus sub-regions for PN 100 rats) were freshly dissected out for quantification of L-arginine and nine downstream metabolites (L-citrulline, L-ornithine, agmatine, putrescine, spermidine, spermine, glutamate, glutamine and γ-aminobutyric acid (GABA)) using liquid chromatography/mass spectrometry and high performance liquid chromatography. For PN 13 rats, the levels of L-arginine and its metabolites were largely unchanged in the whole hippocampus and PFC, except for a significantly increased spermidine level in the PFC in the PCP group. For PN 100 rats, by contrast, the PCP group displayed significantly reduced glutamate and GABA levels in the PFC and hippocampal CA2/3 sub-region, as well as reduced spermidine level in the CA1 sub-region of the hippocampus when compared with the saline group. This study, for the first time, demonstrated that perinatal exposure to PCP during PN 7-11 in rats (equivalent to the third trimester in humans) had prolonged impact on brain arginine metabolism in a region-specific manner. These alterations may contribute to the neurobiological basis for the neurodevelopmental interruption induced schizophrenia-like behavior during the adulthood.

Disclosures: **Y.R. Jing:** None. **L.T. Knox:** None. **H. Zhang:** None. **P. Liu:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.23/E45

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

Support: NIH R15DA034912-01A1

Title: The role of the α7 and α4β2 nicotinic receptors in nicotine sensitization and neural plasticity of adolescent rats neonatally treated with quinpirole: Effects on mTOR

Authors: *D. J. PETERSON[1,2], E. D. CUMMINS[1], S. L. KIRBY[1], M. E. HOWELL[2], C. A. STUART[2], R. W. BROWN[1];
[2]Intrnl. Med. Quillen Coll of Med., [1]East TN State Univ., Johnson City, TN

Abstract: We have established that neonatal treatment with quinpirole, a dopamine D2/D3 agonist, results in increases of dopamine D2 receptor sensitivity throughout the animal's lifetime and has a number of consistencies with schizophrenia. The focus of the current study was to analyze the roles of α7 and α4β2 nicotinic receptors in nicotine sensitization in adolescent male and female rats neonatally treated with quinpirole as well as the roles of the α7 and α4β2 nicotinic receptors were analyzed in their effects on Brain-Derived Neurotrophic Factor (BDNF) and mammalian target of rapamycin (mTOR) in rats neonatally treated with quinpirole and sensitized to nicotine. Animals were neonatally treated with quinpirole or saline from postnatal days (P)1-21. Beginning on P33, animals were ip injected with nicotine (0.5 mg/kg free base) or saline and tested every second day from P33-49. Approximately 15-30 min before the nicotine or saline injection, animals were ip injected with either the α7 nicotinic receptor (nAChR) antagonist methllycacontine (MLA; 2 or 4 mgkg) or the α4β2 nAChR antagonist dihyro-β (DhβE; 1 or 2.5 mg/kg) erythrodine. Brain tissue was taken 1 or 24 h after the last day of testing. Results revealed that neonatal quinpirole enhanced nicotine sensitization and DhβE blocked nicotine sensitization regardless of neonatal treatment and was more effective in blocking sensitization in males versus females. MLA failed to block nicotine sensitization. However, MLA blocked the acute hypoactive response to nicotine in males, and the higher dose of MLA reduced sensitization in males. Neonatal quinpirole sensitized the accumbal BDNF response to nicotine, but neonatal quinpirole resulted in a decrease of mTOR in the nucleus accumbens when brain tissue was taken 24 h after the last nicotine administration. When brain tissue was taken 1 h post-nicotine administration, nicotine resulted in a significant increase of accumbal mTOR which was sensitized by neonatal quinpnirole. These changes in BDNF and mTOR have strong implications towards changes in synaptic growth and strength, and suggest that neonatal quinpirole may result in a significant decrease of mTOR leading to a decrease in synaptic strength, which may be alleviated by nicotine. In conclusion, the α4β2 receptor plays a critical role in adolescent nicotine sensitization. Interstingly, the α7 nAChR appears to be important in

the acute response to nicotine and is more important in nicotine sensitization in males. Both nAChRs appear to be important in accumbal BDNF and their roles will be analyzed in the mTOR response.

Disclosures: D.J. Peterson: None. **E.D. Cummins:** None. **S.L. Kirby:** None. **M.E. Howell:** None. **C.A. Stuart:** None. **R.W. Brown:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.24/E46

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

Support: EDF(AL)

Title: Neonatal functional inactivation of the prefrontal cortex results in increased dopaminergic responses in the core part of the nucleus accumbens to MK801 administration in adult rats

Authors: E. TAGLIABUE, S. EYBRARD, *A. E. LOUILOT;
INSERM U1114- Fac. of Medicine- U.d.S., Strasbourg, France

Abstract: Schizophrenia would result from a defective connectivity between several integrative regions, stemming from neurodevelopmental disturbances (Weinberger and Lipska, 1995; Bullmore et al., 1997; Lewis and Levitt, 2002). Various anomalies reminiscent of early brain development failures have been observed in the patients' left prefrontal cortex (PFC) (Akbarian et al., 1993; Kalus et al., 2000). The existence of a striatal dopaminergic (DA) dysregulation in schizophrenia is generally accepted (e.g. Harrison, 1999; Carlsson et al., 2001). Psychomimetic drugs such as non-competitive NMDA/glutamate receptor 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). The present study was designed to investigate the effects of MK801 (a non-competitive NMDA receptor antagonist) in adult rats on DA responses in the nucleus accumbens (ventral striatum), following an early 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, reversible 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 core part of the nucleus accumbens using *in vivo* voltammetry in freely moving adult rats (11 weeks). DA variations and locomotor responses were measured in parallel. Control animals received a s.c. injection of NaCl (0.9%); MK801 was injected s.c. at 0.1 mg/kg or 0.2 mg/kg. The obtained results were the following : 1) A clear dose effect was observed for the two conditions (PBS and TTX microinjected at PND8); 2) DA increase in the core part of the nucleus accumbens in adult animals after the administration of the highest MK801 dose (0.2 mg/kg) was more elevated in TTX microinjected animals than in PBS microinjected animals. These data suggest that animals microinjected with TTX in the left PFC at PND8 present a more important reactivity to MK801 than PBS microinjected (control) animals. In conclusion, these findings may provide new insights in the pathophysiology of schizophrenia.

Disclosures: **E. Tagliabue:** None. **S. Eybrard:** None. **A.E. Louilot:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.25/E47

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

Support: VIEP-BUAP Grant No. FLAG/SAL12

CONACYT Grant No. 138663

NIH grant AG18440

Title: The neurotrophic factor-like (Cerebrolysin) reduces alterations in myelin and the neurogenesis in dorsal hippocampus induced by the neonatal ventral hippocampal lesion

Authors: *R. A. VAZQUEZ, SR[1], A. ADAME[2], E. MASLIAH[2], G. FLORES[3];
[1]Inst. De Fisiología Benemérita Univ. Autónoma De Puebla, Puebla, Mexico; [2]Dept. of Neurosciences and Pathology, Sch. of Med., Univ. de California San Diego, San Diego, CA; [3]Inst. de Fisiología, Benemerita Univ. Autónoma de Puebla, PUEBLA, Mexico

Abstract: The Neonatal ventral hippocampal lesion model (nVHL) in rats has been widely used as a neurodevelopmental model to mimic schizophrenia-like behaviors. Recently, we reported that the neurotrofic factor-like (cerebrolysin) may have beneficial effects in the management of

the schizophrenia-like behaviors exhibited by nVH-lesions rats. In other hand, Cerebrolysin (CBL) has been shown to increase neurogenesis in models of stroke and AD. CBL is composed of small peptides with activity similar to neurotrophic factors, which may mediate its neurogenic effects. Several reports have been established that newborn neurons migrate a short distance to be integrated into a pre-existing neuronal circuit in the hippocampus. Interestingly, deficits in the expression of doublecortin and proliferating cell nuclear antigen (PCNA) have been described in brain regions in schizophrenia (SZ) in association with abnormalities of cell cycle markers. This study aimed to determine first whether nVH-lesioned rats also exhibited changes in the expression of these cell markers for neurogenesis and the myelin levels (using luxol fast blue stain) in the Hippocampus and second whether Cbl was capable of reducing these alterations in nVHL rats. Our results suggest that nVH-lesion rats shown a reduction in the expression of neuroblast in the DG and a decrease of levels myelin in the Dorsal Hippocampus (DH). Interestingly, Cbl treatment ameliorated these changes and increased the expression of neuroblast (using doublecortin marker) in DG and the levels of myelin in DH of the nVH-lesion rats. In conclusion, this study demonstrates that Cbl promotes neurogenesis in the DG and reduces the alteration in myelin levels induced by the lesion. These findings support the possibility that Cbl may have beneficial effects in the management of schizophrenia symptoms. (Supported by: VIEP-BUAP grant (No. FLAG/SAL12) and CONACYT grant (No. 138663) to G Flores and NIH grant (AG18440) to E Masliah)

Disclosures: **R.A. Vazquez:** None. **A. Adame:** None. **E. Masliah:** None. **G. Flores:** None.

Poster

048. Schizophrenia: Developmental Animal Models

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 48.26/E48

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

Support: NIH grant R15MH098246

Title: Postnatal NMDA receptor antagonism models positive and cognitive, but not negative, symptoms of schizophrenia in adult rats

Authors: *D. C. LOWES, T. A. PAINE;
Oberlin Col., Oberlin, OH

Abstract: Background: Schizophrenia is characterized by positive, negative, and cognitive symptoms. Increasingly, these symptoms are hypothesized to arise from perturbations that occur

during neurodevelopment, although the biological basis of the symptoms is not yet clear. That said, the cognitive symptoms have been linked to dysregulation in GABA neurotransmission in the prefrontal cortex and hippocampus. Furthermore, neonatal exposure to the NMDA receptor antagonist MK-801 has been found to recapitulate positive and cognitive symptoms of schizophrenia and has been found to cause some of the observed changes in GABA transmission. The goal of the current experiment was to determine whether neonatal exposure to a low dose of MK-801 could cause behaviors consistent with the negative symptoms of schizophrenia and could cause changes in GABA system function consistent with that found in schizophrenia. Methods: Male and female rats injected with MK-801 (0.025 mg/kg, s.c.) or saline twice daily from postnatal days (PND) 7-12 were tested for amphetamine-induced hyperlocomotion, sucrose preference, sociality, social preference, and Morris water maze performance in either adolescence (PND 30) or adulthood (PND 90). At the end of testing brains were extracted for Western blot analysis of GABA-related proteins. Results: Postnatal MK-801 administration enhanced amphetamine-induced hyperlocomotion in female adult rats and impaired learning of the Morris water maze in both male and female adult rats. There was no effect on sucrose preference, sociality, or social preference. Preliminary Western blot analysis has found decreased expression of GAD67 in the dorsal hippocampus of male adolescent rats. Discussion: Postnatal exposure to a low dose of MK-801 resulted in mild schizophrenia-like behavioral changes in male and female adult rats. However, there was no change in behaviors that reflect negative symptoms in adult rats. Furthermore, no behavioral changes were observed in rats tested during adolescence. Interestingly, a schizophrenia-like decrease in GAD67 was observed in male adolescent rats suggesting that alterations in GABA function may occur prior to the onset of schizophrenia-like behavioral changes.

Disclosures: **D.C. Lowes:** None. **T.A. Paine:** None.

Poster

048. Schizophrenia: Developmental Animal Models

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Program#/Poster#: 48.27/F1

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

Support: Internal funds, department of psychiatry, Sherbrooke University

Centre des Neurosciences de Sherbrooke

FRQS

Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke

Title: Lack of preventive effect of acute DHA and subacute EPA/DHA combination on PPI disruption in a murine juvenile two-hit model of schizophrenia

Authors: R. GIRARD[1], C. MAURICE-GELINAS[1], C. FAYE[2], G. PARE[1], C. MONPAYS[1], J. DESLAURIERS[3], P. SARRET[1], *S. GRIGNON[1];
[1]Univ. De Sherbrooke, Sherbrooke, QC, Canada; [2]Faculté de Pharmacie, Univ. Paris-Sud, Chatenay Malabry, France; [3]UCSD, San Diego, CA

Abstract: Schizophrenia is a multifactorial, chronic disease in which multiple and intricate pathophysiological factors have been suspected. Among these, oxidative status (OS) disturbances are gaining interest, based on genomic, transcriptomic, biochemical and interventional data. We have recently described and characterized a two hit model (THM) (gestational inflammation followed by adolescent restraint stress (RS)) mimicking some aspects of schizophrenia in juvenile mice, in which behavioural and neurochemical disturbances could be efficiently prevented by the antioxidant lipoic acid1. In clinical studies, ω3 polyunsaturated fatty acids have been shown to efficiently prevent transition to psychosis in an ultra high-risk group, which prompted us to assess their preventive effect in our juvenile THM. **Methods:**. THM: Pregnant C57BL/6 mice were injected with poly-inosinic/cytidylic acide (PolyI:C) 20 mg/kg at gestational day 12 ; their offspring was submitted to RS from PN33 to PN35 (2 hours per day). Prepulse inhibition (PPI) was performed 24h after the last session of RS and the mice were sacrificed for biochemical analyses. Drug administration: Control mice received mineral oil (IP) from PN26 to PN35. The ω3 group received a commercial preparation of PUFA (302.67 mg/kg IP; 20% docosahexaenoic acid (DHA), 30% eicosapentaenoic acid (EPA); content verified by LC-MS), from PN26 to PN35. The DHA group received purified DHA 4 mg/kg (IP) from PN33 to PN35. PN33-35 injections were performed 3h before the RS session. PPI and protein carbonylation were assessed as previously described1. **Results:** As previously described, the THM induced significant PPI deficits, as well as an increase in protein carbonylation, in males. The PPI deficits were not prevented in the ω3 group, and were actually increased in the DHA group in males ($p<0.001$) and females ($p<0.05$). Protein carbonylation was unchanged by DHA or ω3 in the THM x DHA or THM x ω3 groups relative to THM only. **Conclusion:** Under our conditions purified DHA or mixed ω3 did not prevent PPI deficits induced by the THM. Pending further investigation of glutamatergic or dopaminergic markers, timing or duration of administration could account for the discrepancy with existing literature. 1 Deslauriers et al. Neuroscience, 2014. **272**: p. 261-70.

Disclosures: R. Girard: None. **C. Maurice-Gelinas:** None. **C. Faye:** None. **G. Pare:** None. **C. Monpays:** None. **J. Deslauriers:** None. **P. Sarret:** None. **S. Grignon:** None.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.01/F2

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

Support: NSERC

CIHR

Title: Cannabinoid transmission in the ventral hippocampus modulates excitatory neuronal activity in the nucleus accumbens and induces schizophrenia-like disturbances in emotional processing and social cognition behaviors

Authors: *M. LOUREIRO[1], J. RENARD[2], L. G. ROSEN[2], S. R. LAVIOLETTE[2];
[1]Anat. and Cell Biol., [2]Univ. of Western Ontario, London, ON, Canada

Abstract: Cannabis use is a primary risk factor for schizophrenia. Given the exponential increase in marijuana consumption due to legalization, there is an urgent need to better understand the neurobiological mechanisms underlying the effects of cannabinoids on schizophrenia-related emotional processing and social cognition deficits. Cannabinoid type 1 receptors (CB1r) are highly expressed in the mammalian ventral hippocampus (vHipp), a brain region known for its involvement in emotional memory formation and which is profoundly disturbed in schizophrenia. Importantly, the vHipp sends dense excitatory projections to neurons in the nucleus accumbens (NAc), an integrative brain region that is a point of convergence for information arising from cortico-limbic regions, including the amygdala (signalling emotional valence), prefrontal cortex (providing action-outcome information) and vHipp-dependent contextual relevance. The CB1r regulates vHipp output pathways via modulation of inhibitory inputs to excitatory hippocampal output neurons. In addition, previous evidence demonstrates that CB1r activation increases the activity of hippocampal principal neurons. Accordingly, we hypothesized that direct pharmacological activation of intra-vHipp CB1r would increase excitatory vHipp projections to the NAc, thereby activating NAc neuronal activity and potentially modulating mesolimbic-dependent emotional associative memory formation. Using a combination of *in vivo* electrophysiological recordings and behavioural pharmacology in rats, we report that micro-infusion of a direct CB1r agonist (WIN-55,212) into the vHipp, dose-dependently increases spontaneous NAc neuronal firing frequency rates. In addition, we report that intra-vHipp CB1r activation (1) potentiates the emotional salience of normally non-salient context-dependent fear memory, (2) potentiates the reward salience of normally sub-threshold conditioning doses of morphine, measured in an unbiased place conditioning procedure; and (3) induces profound disturbances in social interaction and cognition. Interestingly, these behavioral

effects were completely blocked with direct blockade of AMPA/NMDA receptor transmission, within the NAc, demonstrating a GLUTergic-dependent mechanism underlying these effects. Taken together, these data add new insights into the functional role of hippocampal cannabinoid transmission in the modulation of mesolimbic NAc activity and behavioural dysregulation linked to neuropsychiatric disorders.

Disclosures: **M. Loureiro:** None. **J. Renard:** None. **L.G. Rosen:** None. **S.R. Laviolette:** None.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.02/F3

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

Support: NHMRC Grant APP 1002118

Title: Developmental vitamin D deficiency leads to multiple changes in basal neurotransmitter levels in the neonate rat brain

Authors: ***T. H. BURNE**[1], K. M. TURNER[1], S. ALEXANDER[1], D. EYLES[1], J. J. MCGRATH[1], J. P. KESBY[1,2];
[1]Queensland Brain Inst., Brisbane, Australia; [2]UCSD, San Diego, CA

Abstract: Epidemiological evidence suggests that developmental vitamin D (DVD) deficiency is a risk factor for schizophrenia. We have shown that this is biologically plausible in a rodent model, in which DVD deficiency is associated with changes in brain structure and adult behaviour, and may be associated with altered dopamine and glutamate signalling. Here, we tested the hypothesis that DVD deficiency impacts on multiple neurotransmitter levels in the neonate brain. Sprague-Dawley rats were fed a vitamin D deficient diet (DVD-deficient) or control diet six weeks prior to mating until birth and housed under UVB-free lighting conditions . Basal neurotransmitter levels were then assessed by high-performance liquid chromatography on post-mortem neonate brain tissue. There were significant ($p<0.05$), region-specific increases in the levels of dopamine in the basal ganglia and noradrenaline in the hippocampus of newborn DVD-deficient rats. Whereas, glutamine levels were consistently reduced ($p<0.05$) by 12-24% in most brain regions examined. Our results confirm that DVD deficiency leads to changes in multiple neurotransmitter systems in a regionally-specific manner in the neonate brain. These data suggest that the glutamate-glutamine cycle is altered and may underlie the alterations in

dopamine and glutamate signalling previously described in DVD-deficient rats. Taken together, these data suggest that DVD deficiency alters neurotransmitter systems relevant to schizophrenia in the developing rat brain.

Disclosures: T.H. Burne: None. **K.M. Turner:** None. **S. Alexander:** None. **D. Eyles:** None. **J.J. McGrath:** None. **J.P. Kesby:** None.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.03/F4

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

Title: Effects of the antioxidant n-acetyl cysteine on behavioral and neurophysiological deficits induced by developmental NMDA-R antagonism

Authors: *A. J. PHENSY[1], C. DRISKILL[2], V. JEEVAKUMAR[2], S. BREWER[2], C. DE LA HOZ[2], S. KROENER[2];
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Abstract: The NMDA-hypofunction theory of schizophrenia suggests that schizophrenia is associated with a loss of NMDA receptors, specifically on corticolimbic parvalbumin (PV)-expressing GABAergic interneurons, leading to disinhibition of pyramidal cells and cortical desynchronization. We recently characterized the behavioral and physiological effects of developmental NMDAR antagonism model in which mice receive ketamine (KET) injections on postnatal days (PND) 7, 9 and 11 (Jeevakumar and Kroener, Cereb. Cortex, 2014; Jeevakumar et al., Behav. Brain Res. 2015). We found that sub-chronic developmental KET treatment results in a loss of PV expression in the medial prefrontal cortex; reduced cognitive flexibility in an attentional set-shifting task; an unexpected upregulation of NMDAR responses in PV expressing interneurons in layer 5, and changes in the excitation-inhibition balance as measured by recordings of spontaneous and miniature EPSCs and IPSCs, respectively. The loss of PV expression following subchronic NMDA antagonism appears to be the result of oxidative stress, involving the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Here we investigated whether the antioxidant N-acetyl cysteine (NAC) can prevent the KET-induced loss of PV expression as well as the resultant behavioral and physiological changes. Groups of ketamine- and saline-treated animals were given subcutaneous injections of either NAC or saline between PND 5-21. After weaning, animals in the NAC treatment groups

continued drinking NAC in their drinking water through adulthood. In adult NAC-treated animals levels of PV expression in the mPFC remain elevated close to control levels, whereas the numbers of PV+ cells in animals that received saline injections after KET treatment sharply declined. NAC also improved performance on the attentional set-shifting task in KET-treated animals. In agreement with our previous findings, developmental KET treatment decreased the frequency of miniature IPSCs at layer 2/3 pyramidal cells, suggesting reduced GABAergic inhibition. The reduction in mIPSCs onto pyramidal cells in KET-treated mice was mirrored by an increase in spontaneous excitatory postsynaptic currents onto PV+ interneurons in the same layer, further suggesting persistent cortical disinhibition. Both of these KET-induced changes in synaptic transmission were prevented by the NAC treatment. Ongoing experiments investigate whether KET treatment also alters the NMDA:AMPA ratio in layer 2/3 interneurons, and whether these changes can be prevented by NAC, thus preserving normal prefrontal cortical synchronization and cognitive abilities.

Disclosures: **A.J. Phensy:** None. **C. Driskill:** None. **V. Jeevakumar:** None. **S. Brewer:** None. **C. de la Hoz:** None. **S. Kroener:** None.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.04/F5

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

Support: NH104344

Title: Scopolamine produces greater attenuation of forced swim immobility in mice housed in a short-active photoperiod

Authors: *Z. A. COPE[1], D. DULCIS[1], J. W. YOUNG[1,2];
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Abstract: Seasonal variation in day-length is believed to increase risk for relapse to depression or mania in individuals with bipolar disorder (Wang et al. J. Psych. Practice 2013). This risk can be partially attenuated with treatments such as social rhythm therapy or timed light exposure (Dallaspezia et al. Informa Expert Reviews 2011). Housing normal rats in altered photoperiods is sufficient to produce behaviors consistent with mania- or depression-relevant behaviors. That is, rats housed in a long-active (LA) photoperiod (19 hours dark, 5 hours light) exhibited increased

elevated plus maze open arm entries, while those housed in a short-active (SA) photoperiod (5 hours dark, 19 hours light) exhibited increased immobility in the forced swim task (FST). These behaviors were associated with respective switches in expression of tyrosine hydroxylase (TH) or somatostatin (SST) in neurons of the periventricular nucleus (PVN) that are coincident with decreases (LA) or increases (SA) in corticosterone and corticotrophin releasing factor (CRF, Dulcis et al. Science 2013). Preliminary experiments from our lab have behaviorally replicated these findings in mice. Given that the immobility resulting from a SA photoperiod was associated with increased SST in PVN neurons and subsequent increases in CRF, we hypothesized that the resulting depressed phenotype is likely to result from elevated CRF stimulated acetylcholine (ACh). To test this hypothesis, male C57BL/6J mice were housed in either a normal (12 hours light, 12 hours dark) or SA photoperiod for two weeks before testing in the FST. Mice were injected (s.c.) with one of three doses of the muscarinic acetylcholine receptor antagonist scopolamine 10 min before testing. All doses of scopolamine significantly reduced immobility in the FST compared to saline. Interestingly, SA mice appeared more sensitive to the effects of scopolamine at the lowest dose administered as indicative of a larger effect size. These preliminary findings indicate that extended exposure to a SA photoperiod modified cholinergic signaling in these animals, supporting our hypothesis. Hence, SA photoperiod may induce a depressive-like state by altering the balance of SST expression in PVN neurons, thereby increasing CRF release, and ultimately impinging on normal ACh function.

Disclosures: **Z.A. Cope:** None. **D. Dulcis:** None. **J.W. Young:** None.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.05/F6

Topic: C.19. Drug Discovery and Development

Title: SGE-516, a novel allosteric modulator of synaptic and extra-synaptic GABAA receptors, has an anxiolytic-like effect *in vivo*

Authors: *R. S. HAMMOND, M. A. ACKLEY, C. MACIAG, G. M. BELFORT, G. MARTINEZ-BOTELLA, F. G. SALITURO, J. J. DOHERTY, A. J. ROBICHAUD;
Sage Therapeut., Cambridge, MA

Abstract: Neurosteroids such as allopregnanolone, a reduced metabolite of progesterone, have been associated with affective disorders including anxiety and depression. Decreased serum or CSF allopregnanolone levels have been observed in depressed patients, supporting the

hypothesis that the down regulation of neurosteroids may be a risk factor for the development of affective disorders. Consistently, allopregnanolone administration produces antidepressant-like and anxiolytic-like effects in rodents. Unlike the vast majority of benzodiazepines, neurosteroids are positive allosteric modulators of both synaptic and extrasynaptic GABAA receptors and may provide a differentiated therapeutic profile in affective disorders. However, these endogenous neurosteroids have limited oral bioavailability and rapid clearance, limiting their utility as oral therapeutics. We have therefore designed novel neurosteroid modulators of synaptic and extrasynaptic subunit-containing GABAA receptors with improved pharmacokinetic properties. SGE-516 is an exemplar with pre-clinical anxiolytic-like properties at exposures that do not cause sedation. SGE-516 potently potentiates GABA currents at synaptic and extra-synaptic receptors, respectively composed of either $\alpha1\beta2\gamma2$ or $\alpha4\beta3\delta$ subunits, and was designed to have pharmacokinetic properties that are appropriate for oral administration. The anxiolytic potential of SGE-516 was assessed following acute dosing in two rodent models, the mouse elevated plus maze and the rat Vogel conflict test. In the elevated plus maze, SGE-516 increased the number of entries and time spent in open arms, an effect consistent with anxiolytic-like efficacy and similar to diazepam (2 mg/kg, IP). At the minimal effective dose (MED) of 3 mg/kg (IP), the total exposure was 1,182 nM in plasma and 1,479 nM in brain. SGE-516 also had an effect consistent with anxiolytic efficacy in the rat Vogel conflict test. SGE-516 increased the number of shock episodes and punished licks in 10 minutes. At the MED of 3 mg/kg (IP), the total exposure was 760 nM and 1082 nM in plasma and brain respectively. At similar plasma and brain exposures SGE-516 (3 mg/kg, IP) did not reduce locomotor activity in the open field or impair motor coordination on the rotarod in mice. These data suggest that SGE-516, a novel positive allosteric modulator of GABAA receptors, has anxiolytic-like effects in two different models. Compounds such as SGE-516 may therefore represent a novel class of molecules with potential therapeutic use as non-sedating anxiolytics.

Disclosures: **R.S. Hammond:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **M.A. Ackley:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **C. Maciag:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **G.M. Belfort:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **G. Martinez-Botella:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **F.G. Salituro:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **J.J. Doherty:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **A.J. Robichaud:** A. Employment/Salary (full or part-time):; Sage Therapeutics.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.06/F7

Topic: C.19. Drug Discovery and Development

Title: New insights for development of a selective muscarinic M1 agonist

Authors: *C. H. CROY[1], D. EVANS[2], B. LIU[3], M. BURES[3], E. COLVIN[4], A. MOGG[4], L. BROAD[4], P. GOLDSMITH[2], C. FELDER[1];
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Abstract: The observation that cholinergic deafferentation of circuits projecting from forebrain basal nuclei to frontal and hippocampal circuits occurs in Alzheimer's disease has led to drug-targeting of muscarinic M1 receptors to alleviate cognitive symptoms. The high homology within the acetylcholine binding domain of this family however has made receptor-selective ligand development challenging. This work presents the structure-activity-relationship study findings for M1-selective ligand and its functional characterization as an orthosteric ligand. Additionally, homology modeling work will illustrate that the binding site of this compound spans from the acetylcholine pocket to the extracellular loops of the receptor, a common allosteric vestibule for the muscarinic protein family. Altogether data presented will support that this M1-selective ligand represents a growing class of multi-topic ligands which interact with the receptors in both the ortho- and allosteric binding sites, but which exert their activation mechanism as an orthosteric ligand.

Disclosures: **C.H. Croy:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **D. Evans:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **B. Liu:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **M. Bures:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **E. Colvin:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **A. Mogg:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **L. Broad:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **P. Goldsmith:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **C. Felder:** A. Employment/Salary (full or part-time):; Eli Lilly and Company.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.07/F8

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Enhanced endocannabinoid signaling amplifies neural activity flow through the hippocampal trisynaptic circuit and promotes safety learning

Authors: J. STEPAN[1], V. MICALE[2], *J. DINE[1], C. WOTJAK[1], M. EDER[1];
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Abstract: Anxiety disorders like post-traumatic stress disorder, panic disorder, and phobias are frequently diagnosed and represent a relevant global health problem. Exposure-based therapies in combination with drug administration are widely used as treatment for most of these brain based diseases. Classical drugs like benzodiazepines hamper therapeutic effects by severely disturbing amnestic functions. Recently, substances targeting the endocannabinoid system were found to be effective anxiolytics without detrimental side effects on memory function. However, the alterations in millisecond-scale brain circuit dynamics that might underlie their therapeutic actions remain elusive. Here, enabled by a recently developed voltage-sensitive dye imaging assay in mouse brain slices, we compared the impact of diazepam and the cannabinoid neurotransmission enhancer AM404 on neuronal activity propagations through the entire trisynaptic circuitry of the hippocampus ("HTC": perforant path-dentate gyrus-area CA3-CA1 output subfield). Bath applied diazepam (1 μM) led to markedly weakened activity propagations ("HTC-Waves"), which coincided with impaired safety learning. In contrast, bath applied AM404 (10 μM) caused an amplification of HTC-Waves in CA regions, and promoted safety learning. These effects were absent in D1CB1-KO mice. Collectively, we demonstrate bidirectional effects of two anxiolytic drugs on the hippocampal network that might underlie their opposing effects on memory function during exposition-based therapies.

Disclosures: J. Stepan: None. **V. Micale:** None. **J. Dine:** None. **C. Wotjak:** None. **M. Eder:** None.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

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

Support: NARSAD Young Investigator Award to DPG

VA Career Development Award (CDA-2) IK2BX001650 to DPG

NIAAA Grant RO1AA021662 to SCP

VA Merit Grant I01BX000143 to SCP

VA senior research career scientist award to SCP

Title: Cannabinoid Receptor 1 interaction with Epigenetic mechanisms

Authors: J. S. DESAI[1,2], H. KUSUMO[1,2], S. C. PANDEY[1,2], *D. P. GAVIN[1,2];
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Abstract: Background: Cannabis use in adolescence is a recognized environmental risk factor that decreases the age of schizophrenia onset and increases the severity of psychosis. On the other hand, schizophrenia patients abuse cannabinoids at a higher rate than nonpsychiatric controls. It is unclear whether cannabinoid use by schizophrenia patients is a precipitating factor for enduring psychotic symptoms potentially maintained through epigenetic mechanisms or is a means of self-medication. Increases in restrictive chromatin inducing-enzymes leading to decreases in Glutamate decarboxylase 1 (GAD1) and Brain-derived neurotrophic factor (BDNF) expression have been reported in schizophrenia. In the current study we hypothesized that cannabinoid 1 receptor (Cb1) modulation would alter epigenetic parameters in neurons, thereby affecting the expression of these genes. Methods: We treated mouse E18 primary cortical neuron cultures with the Cb1 agonist, arachidonyl-2-chloroethylamide (ACEA). We measured changes in epigenetic gene expression via qRT-PCR, as well as potential downstream schizophrenia candidate genes in neuron culture. Western blot was used to quantify Cb1 agonist induced changes in the 'open' chromatin mark histone H3 lysine 79 dimethylation (H3K79me2). Results: In primary neuron cultures, ACEA increases the mRNA expression of the enzyme that catalyzes the addition of methyl groups to H3K79, Dot1l, in a dose and time dependent manner with maximal increase at 0.1 μM at 6 hours. This increase in Dot1l corresponds to increases in H3K79me2 protein levels, as well as Gad1 and BdnfIXa mRNA. At ACEA doses exceeding 1 μM, enzymes that catalyze the transcription silencing mark dimethylation of lysine 9 of histone 3 (H3K9me2), Glp, G9a, and Setdb1, as well as DNA methylating enzymes, Dnmt1 and Dnmt3a are induced. Discussion: This is the first study to our knowledge to examine the effects of cannabinoids on epigenetic parameters in neurons. We find that low doses of a Cb1 agonist induce factors that 'open' chromatin, while high doses induce expression of chromatin restricting factors. Dysregulation of epigenetic mediators affecting Gad1 and Bdnf gene expression have been repeatedly implicated in schizophrenia. Based on these results we speculate that altered cannabinoid system functioning, whether due to substance abuse or endogenous differences in the endocannabinoid system, may contribute to the epigenetic and gene expression differences

observed in schizophrenia. [Supported by NIAAA, Department of Veterans Affairs Merit and Senior Career Scientist Awards to SCP and VA career development award (IK2BX001650) to DPG].

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Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.09/F10

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

Support: Brain Canada Multi-Investigator Research Initiative

Title: The T-type calcium channel antagonist Z944 disrupts prepulse inhibition in three strains of rats

Authors: *J. G. HOWLAND[1], W. N. MARKS[2], Q. GREBA[2], S. M. CAIN[3], T. P. SNUTCH[3]; [1]Physiol., Univ. Saskatchewan, Saskatoon, SK, Canada; [2]Physiol., Univ. of Saskatchewan, Saskatoon, SK, Canada; [3]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Low voltage-activated T-type calcium channels are widely expressed in the brain. The electrophysiological characteristics of T-type calcium channels and their role in brain diseases such as absence epilepsy and neuropathic pain have been studied extensively. However, less is known regarding the involvement of T-type channels in cognition and behavior. Sensorimotor gating is a basic process whereby the brain filters incoming stimuli to enable appropriate responding in sensory rich environments. Prepulse inhibition (PPI) is a common measure of sensorimotor gating used in studies employing both humans and animals because of its cross species validity, face and predictive validity, ease of implementation, and reliability. The regulation of PPI involves a network of limbic, cortical, striatal, pallidal, and pontine brain areas, many of which show high levels of T-type calcium channel expression. Therefore, we tested the effects of blocking T-type calcium channels on PPI with the potent and selective T-type antagonist Z944 (0.3, 1, 3, 10 mg/kg; i.p.) in adult Wistar rats and two related strains, the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and Non-Epileptic Control (NEC). All rats were carefully handled during the week prior to the PPI test. PPI was tested using a protocol that varied prepulse intensity (3, 6, and 12 dB above background) and prepulse-pulse interval (30, 50, 80, 140 ms). Wistars showed significantly higher startle responses to the pulse alone during the test session than the other two strains; Z944 did not significantly affect startle at the doses tested.

All three strains had similar levels of PPI when injected with vehicle. Z944 dose-dependently disrupted PPI in all strains for both long (50, 80, 140 ms) and short intervals (30 ms) with the most potent effect observed following treatment with the higher doses. These findings suggest that T-type calcium channels contribute normal patterns of brain activity that regulate PPI. Given that PPI is disrupted in psychiatric disorders including schizophrenia, Huntington's disease, and obsessive compulsive disorder, future experiments that test the specific brain regions involved in the regulation of PPI by T-type calcium channels may help inform therapeutic development for those suffering from sensorimotor gating impairments.

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Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.10/F11

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

Support: CIHR-MOP-300152

HH Jasper Fellowship

Title: Tardive dyskinesia induced by prolonged antipsychotic treatments in a non-human primate model is associated with Akt/GSK-3ß kinase activities

Authors: *G. A. HERNANDEZ[1], S. MAHMOUDI[2], M. CYR[4], P. J. BLANCHET[3], D. LÉVESQUE[2];
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Abstract: Tardive dyskinesia (TD) is a delayed and potentially irreversible motor complication arising in patients chronically exposed to dopamine receptor antagonists. Typical antipsychotic drugs, metoclopramide and several atypical drugs are associated with generation of TD. But, the pathophysiology of TD remains elusive and difficult to treat. Antipsychotic drugs modulate multiple kinase pathways, but their involvement in TD is unknown. To investigate the neurochemical basis of TD, we exposed capuchin (Cebus apella) monkeys to prolonged haloperidol (N=11) or clozapine (N=6) treatments. Six untreated animals were used as controls.

Five haloperidol-treated animals developed mild TD movements similar to those found in humans. No TD was observed in the clozapine group. We measured ERK1/2, GSK-3β and Akt activities with phospho[Thr202/Tyr204]-p44/42 (pERK1/2), phospho[Ser9]-GSK-3β (pGSK-3β) and phospho[Ser473]-Akt (pAkt) specific antibodies by Western blots. Relative pERK1/2, pGSK-3β and pAkt levels were calculated from respective total kinase levels. Haloperidol, but not clozapine, strongly enhanced pERK1/2 immunoreactivity in the putamen. Nonetheless, both dyskinetic and non dyskinetic animals showed similar pERK1/2 levels. On the other hand, haloperidol reduced putamen pAkt and pGSK-3β immunoreactive signals. Interestingly, only haloperidol-treated monkeys that did not develop dyskinesia have reduced pAkt and pGSK-3β levels, as compared to dyskinetic animals, and pAkt levels nicely correlated with dyskinetic scores ($r2 = 0.916$; p value = 0.011). These results suggest that a reduced Akt/GSK-3β activity minimizes the risk of haloperidol-induced TD.

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Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.11/F12

Topic: C.19. Drug Discovery and Development

Title: Preclinical development of a novel class of competitive inhibitors of the Glycine Transporter-1 (GlyT-1) for the treatment of cognitive deficits

Authors: *N. MOORE[1], A. RASSOULPOUR[1], C. CIOFFI[2], S. LIU[3], P. GUZZO[3], M. LUCHE[2], A. MHYRE[4];
[1]Brains On-Line LLC, South San Francisco, CA; [2]AMRI, Albany, NY; [3]Concerted Therapeut. Inc., Albany, NY; [4]Fred Hutchinson Cancer Res. Ctr., Seattle, WA

Abstract: Treatments for cognitive deficits associated with Alzheimer's disease, schizophrenia and aging remain a major unmet need. Recent evidence suggests that the glycine transporter-1 (GlyT-1) may be a promising target. Behavioral data from both rodent and primate studies demonstrate positive effects of non-competitive GlyT-1 inhibitors in a range of cognitive models; however, clinical evidence is less compelling. Some studies have suggested that an optimum level of occupancy is necessary to see positive effects. We have speculated that this narrow therapeutic window may in part be due to the non-competitive nature of the binding to the GlyT-1 site. We have developed a novel series of competitive GlyT-1 inhibitor compounds.

AMR-GLY-6 and AMR-GLY-10 represent examples of the series that have been shown to be competitive and selective. Here we present data demonstrating that *in vivo* these compounds possess GlyT-1 inhibitor-like properties in both rats and non-human primates leading to indications of cognitive enhancement with no adverse side effects. AMR-GLY-6 and AMR-GLY-10 were shown to block the reuptake of [14C]-glycine by hGlyT-1a expressed in JAR cells (IC50 0.67nM and 1.06nM respectively) with negligible activity at GlyT-2 or other off-target sites. The binding to the GlyT-1 site was competitive in nature. *In vivo*, the compounds were evaluated using both microdialysis (prefrontal cortex [Pfc] glycine levels) and measurements of cerebrospinal fluid (CSF) glycine coupled with compound exposure in brain and plasma following acute oral administration in rats. Both compounds produced significant dose-related elevations in Pfc glycine which translated into dose-dependent increases in CSF glycine levels. Upon repeat dosing AMR-GLY-6 maintained elevated CSF glycine levels throughout a five day dosing period without producing adverse effects seen with earlier non-competitive GlyT-1 inhibitors. The effect was reversible with glycine levels rapidly returning to normal once dosing was stopped. In cynomolgus monkeys, AMR-GLY-10 produced dose-related increases in CSF glycine levels and improvements in cognitive functioning in an objective retrieval task in normal primates without producing adverse effects. This data demonstrates that competitive inhibition of the GlyT-1 site may offer a novel approach to improve cognitive functioning. Furthermore the use of CSF glycine measurement coupled with brain and plasma compound analysis could be an inexpensive biomarker for dose selection in clinical studies.

Disclosures: **N. Moore:** A. Employment/Salary (full or part-time):; Brains On-line LLC. **A. Rassoulpour:** A. Employment/Salary (full or part-time):; Brains On-line LLC. **C. Cioffi:** A. Employment/Salary (full or part-time):; AMRI. **S. Liu:** A. Employment/Salary (full or part-time):; Concerted Therapeutics Inc. **P. Guzzo:** A. Employment/Salary (full or part-time):; Concerted Therapeutics Inc. **M. Luche:** A. Employment/Salary (full or part-time):; AMRI. **A. Mhyre:** A. Employment/Salary (full or part-time):; Fred Hutchinson Cancer Research.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.12/F13

Topic: C.19. Drug Discovery and Development

Support: All authors are employees of AbbVie

The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in data interpretation, review and publication approval.

Title: *In vivo* evaluation of target occupancy by positron emission tomography (PET) and pharmacodynamic assessment of ABT-419, a glycine transporter-1 (GlyT1) inhibitor in non-human primates (NHP)

Authors: *A. M. BASSO[1], R. RAJAGOVINDAN[1], J.-Q. WANG[1], A. E. TOVCIMAK[1], Y. LAO[2], C. KALVASS[2], M. J. VOORBACH[1], A. GIAMIS[1], D. REUTER[1], S. CASSAR[1], P. JACOBSON[1], R. CARR[3], E. VAN DER KAM[4], B. BEHL[4], G. B. FOX[1], J. D. BEAVER[1]; [1]Translational Sciences-Imaging, [2]Preclinical Pharmacokinetics, [3]Translational Modeling, AbbVie, North Chicago, IL; [4]Neurosci. Discovery, AbbVie, Ludwigshafen, Germany

Abstract: GlyT1 regulates synaptic glycine concentration in the forebrain. Since glycine is a co-agonist of the NMDA receptor, GlyT1 inhibition has been proposed as a potential treatment to reverse the NMDA receptor hypofunction suggested to be affected in schizophrenia. ABT-419 is a novel, potent (Ki = 1 nM), selective and competitive GlyT1 inhibitor. The goal of this study was to determine the relationship between ABT-419 plasma concentrations and brain GlyT1 occupancy and changes in CSF glycine in NHP following administration of ABT-419. Occupancy of GlyT1 was measured by dynamic PET scans with arterial sampling using [^{18}F]CFPyPB, a previously reported radiotracer. Scanning was conducted at baseline and following different doses of ABT-419 infused i.v. over a 3-h period or given as i.v. bolus. [^{18}F]CFPyPB was administered as i.v. bolus 1-h after the start of ABT-419 infusion and was followed by 2-h dynamic scans. Time course studies evaluated the relationship between GlyT1 occupancy and ABT-419 plasma concentrations as well as changes in CSF glycine, ABT-419 plasma and CSF concentrations following ABT-419 administration. Glycine and ABT-419 concentrations were determined by LC-MS/MS. The highest uptake of [^{18}F]CFPyPB and GlyT1 occupancy with ABT-419 was observed in brainstem, pons, midbrain, cerebellum and thalamus. Dose-dependent target occupancy was achieved, reaching full occupancy (>90%) at 5 mg/kg of ABT-419. A counter-clockwise hysteresis was observed for ABT-419 plasma pharmacokinetic profile and the brain GlyT1 target occupancy, with time dependent EC_{50} values and lower EC_{50} at the later scan time points. ABT-419 displayed a longer half-life in CSF (13 h) as compared to plasma (3 h). Administration of ABT-419 resulted in a significant increase of glycine in CSF which demonstrated a direct relationship with GlyT1 target occupancy and ABT-419 CSF concentration. PET and pharmacodynamic data suggest prolonged GlyT1 brain occupancy and indirect kinetics with respect to ABT-419 plasma concentrations but a direct relationship with respect to ABT-419 CSF concentration. Therefore, occupancy analysis using a direct ABT-419 plasma levels/ target occupancy relationship is not appropriate and further indirect kinetic modeling is needed. NHP *in vivo* PET studies with ABT-419 supported the prediction of pharmacologically active doses in humans and initial planning for a first-in-human study (Brain PET 2015, poster presentation). Current data strongly support the use of PET target occupancy

and pharmacodynamic biomarker studies for translational purpose in clinical development. All authors are AbbVie employees. Financial support was provided by AbbVie.

Disclosures: **A.M. Basso:** A. Employment/Salary (full or part-time):; AbbVie. **R. Rajagovindan:** A. Employment/Salary (full or part-time):; AbbVie. **J. Wang:** A. Employment/Salary (full or part-time):; AbbVie. **A.E. Tovcimak:** A. Employment/Salary (full or part-time):; AbbVie. **Y. Lao:** A. Employment/Salary (full or part-time):; AbbVie. **C. Kalvass:** A. Employment/Salary (full or part-time):; AbbVie. **M.J. Voorbach:** A. Employment/Salary (full or part-time):; AbbVie. **A. Giamis:** A. Employment/Salary (full or part-time):; AbbVie. **D. Reuter:** A. Employment/Salary (full or part-time):; AbbVie. **S. Cassar:** A. Employment/Salary (full or part-time):; AbbVie. **P. Jacobson:** A. Employment/Salary (full or part-time):; AbbVie. **R. Carr:** A. Employment/Salary (full or part-time):; AbbVie. **E. van der Kam:** A. Employment/Salary (full or part-time):; AbbVie. **B. Behl:** A. Employment/Salary (full or part-time):; AbbVie. **G.B. Fox:** A. Employment/Salary (full or part-time):; AbbVie. **J.D. Beaver:** A. Employment/Salary (full or part-time):; AbbVie.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.13/F14

Topic: C.19. Drug Discovery and Development

Title: Known CNS medications produce distinct behavioral profiles in a larval zebrafish high content phenotypic screen

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Abstract: Behavioral phenotypes are powerful tools for drug discovery and the analysis of neuronal signaling. Given the heterogeneous nature of many CNS diseases, phenotypic approaches are once again gaining acceptance as an effective method for identifying new CNS medicines. The goal of the present study was to assess and characterize the behavioral effects of a range of approved CNS drugs from multiple therapeutic classes in the Teleos zebrafish phenotypic profiling platform. Fertilized eggs (up to 20,000 embryos per day) were collected from group mating's of wild-type zebrafish. Larvae were raised until 7 days post fertilization and then distributed into the wells of clear 96-well plates. Test drugs, including DMSO control, were

added to each well at various concentrations and their effects on zebrafish behaviors were recorded using a digital video of the entire 96-well plate. We found that compounds in the same pharmacological class generate similar phenotypic effects, whereas compounds in different classes generate dissimilar effects. These behavioral data can be represented geometrically as points in a high dimensional space, and therefore time series corresponding to compounds in the same pharmacological class cluster together. Such clustering allowed us to visualize the relations among all known compounds in the screen. In this way it is possible to distinguish between active drugs from different therapeutic classes and, in many cases, distinguish between drugs from the same therapeutic class that have distinct mechanisms of action. Together, these data suggest that behavioral profiling in zebrafish is an effective way to identify and classify CNS compounds for research and therapy.

Disclosures: G. Carey: A. Employment/Salary (full or part-time):; Galen Carey, PhD Teleos Therapeutics, Tama Evron, PhD Teleos Therapeutics, Andrea Velenich, PhD Teleos Therapeutics, Dennis Lensen, PhD Teleos Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Adam Rosenberg, Dave Kokel PhD, Galen Carey PhD, Randall T Peterson. **T. Evron:** A. Employment/Salary (full or part-time):; Teleos Therapeutics. **A. Velenich:** A. Employment/Salary (full or part-time):; Teleos Therapeutics. **D. Lensen:** A. Employment/Salary (full or part-time):; Teleos Therapeutics. **D. Kokel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Teleos Therapeutics. **R.T. Peterson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Teleos Therapeutics.

Poster

049. Schizophrenia: Molecular and Cellular Mechanisms

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 49.14/F15

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

Support: NIH Grant MH097997

Title: Involvement of long noncoding RNA- NEAT1 and paraspeckles proteins in oligodendrogenesis - relevance to schizophrenia

Authors: *V. HAROUTUNIAN[1], P. FAM[2], W. TAN[3], M. PLETNIKOV[4], S. NAKAGAWA[5], P. KATSEL[3];

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Abstract: Long noncoding RNA - NEAT1 is a structural component of paraspeckles, subnuclear bodies in interchromatin region that control sequestration of paraspeckle proteins. Paraspeckle function in neural cell types has not been studies extensively. Microarray studies of multiple cortical regions from individuals with schizophrenia (SZ) showed strong downregulation of NEAT1 levels across 15 different brain regions compare to controls. Gene clustering placed NEAT1 in proximity to myelin-specific genes. Myelin-related gene/protein expression decline is among the most corroborated observations in the pathophysiology of SZ. We hypothesize that NEAT1 dysregulation is associated with oligodendrocyte (OLG) function. We tested this hypothesis in three different mouse models: 1) demyelination model, quaking mouse (Qke5); 2) increased OLG proliferation model, forebrain restricted expression of mutant hDISC1 mouse and 3) NEAT1 knock out model. We examined gene expression of lncRNA - NEAT1, paraspeckle protein - PSPC1, and the molecular determinants of different glial cell types. We found: i) Myelin specific genes (MAG, CNP, Sox10, PLP and MBP) and astrocyte marker, ALDH1L1 were dramatically downregulated in white matter of Qke5 mice. Similarly, the levels of NEAT1 and PSPC1 were significantly decreased (near 6 fold and 1.4 fold, respectively) in white matter of Qke5 mice. ii) Forebrain-restricted overexpression of mutant hDISC1 mice had the profound effect on NEAT1 and PSPC1 expression throughout development and in adult mice, similarly to the effect on myelin-related genes. iii) Myelin specific genes (MAG, CNP, Sox10 and CLDN11), OLG progenitors (PDGFRA and CSPG2) and astrocyte marker, ALDH1L1, were significantly downregulated in frontal cortex of adult NEAT1 KO mice. Neuronal ENO2, or a microglia-specific marker, CD86 were unchanged. These findings suggest strong involvement of NEAT1 and paraspeckle protein PSPC1 in oligodendrogenesis including early development and throughout the lifespan and bring into prominence the role of paraspeckles and NEAT1 in the pathophysiology of SZ.

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Poster

050. Alcohol Seeking, Reward, and Relapse

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 50.01/F16

Topic: C.17. Drugs of Abuse and Addiction

Support: Alkoholitutkimusäätiö

Title: Using IntelliCage to study alcohol drinking behavior in mice

Authors: *M. VEREMIEVA[1], T. PIEPPONEN[2], J.-O. ANDRESSOO[1], V. VOIKAR[3], M. AIRAVAARA[1];
[1]Inst. of Biotech., [2]Fac. of Pharm., [3]Neurosci. centre, Univ. of Helsinki, Helsinki, Finland

Abstract: Background: Ethanol is the most widely misused abused substance leading to alcohol-related disorders. A big challenge in a treatment of alcohol addiction is relapse that can occur even after long period of abstinence. Unlike in rats, mouse models of alcohol craving are not well established and the number of gene manipulated mouse strains offers the possibility for investigating biology of alcohol craving. We aimed at developing a novel model of alcohol craving in mice in the group housed environment with minimal handling by human during experiment. Methods: C57BL/6 mice were used and individually recognized in the IntelliCage by subcutaneously implanted transponders. The mice were placed into IntelliCages in groups of 8-10 and had access to either 12% ethanol in 0.5% of saccharin, 12% ethanol, or 0.5% saccharin and water. In the training phase, the mice had access to bottles in two corners of the cage. One side of the corner contained CS+ treatment and the other side water. Nosepoke to CS+ side was signaled by green light (conditioned stimulus). Training lasted for 10 days and then the mice were transferred to regular cages. After withdrawal period (1-10 days) the experimental procedures were the same, but there was no liquid available. Results: During alcohol drinking training the number of visits was increased in alcohol+saccharin group and decreased in alcohol group as compared to control (water only) group. The number of nosepokes was increased in alcohol+saccharin group during the 10-day training compared to any other group. The number of licks was higher in saccharin group in comparison to alcohol, alcohol+saccharin and water groups. After withdrawal the number of nosepokes in correct corner and number of conditioned licks were higher only in alcohol+saccharin group on both withdrawal days 1 and 10. Conclusion: The mice did not develop high voluntary preference for alcohol in home-cage and social environment. However, increased activity in the corners with light signal and especially distinct changes after withdrawal period suggest development of alcohol craving. Moreover, only sweetened alcohol produced significant craving after withdrawal. In conclusion, new methodology will give us an opportunity to study alcohol drinking behavior and alcohol craving after withdrawal in mice in automated and time saving manner.

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Poster

050. Alcohol Seeking, Reward, and Relapse

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 50.02/F17

Topic: C.17. Drugs of Abuse and Addiction

Title: Ethanol self-administration and decision making in a free reward choice task in the rat

Authors: J. J. MCGRAW[1], L. C. ZONA[2], *H. C. CROMWELL[1];
[1]Bowling Green State Univ., Bowling Green, OH; [2]Bowling Green State Unviersity, Bowling Green, OH

Abstract: Drinking alcohol can affect many aspects of decision making. Discriminating and choosing between reward outcomes, developing preferences, and updating reward information that is susceptible to change (i.e., incentive contrast; Flaherty, 1996) are important processes of choice behavior that have been studied using animal models of decision making. For example, alcohol exposure has been known to alleviate negative contrast caused by downshifts in sucrose reward (Becker & Flaherty, 1982). However, the long-term effects of alcohol on incentive contrast in accordance with other measures of motivation have not been investigated. Previous studies have used reward discrimination tasks such as mazes and runways and operant tasks such as lever pressing to study reward-based decision making. Despite success, these traditional methods rely heavily on learning, offer limited space, and force behavior which restricts the ability to make choices based solely on reward outcomes. Our paradigm examines the effect of a history of voluntary alcohol consumption on free reward choice in a novel apparatus and how reward processing may be altered by chronic self-administration of alcohol by rats. A novel 3-box design was used to assess how these components of decision making may interact to motivate unrestricted choice behavior in rats. The present study investigated acute and chronic effects of alcohol on normal outbred Sprague-Dawley rats trained to drink moderate amounts of alcohol voluntarily for a chronic period concurrent with 6 weeks of behavior testing. We offered two different reward outcomes each week to examine how behavior toward changing reward scenarios is impacted by a history of alcohol consumption. A modified sucrose-fade procedure was used to establish significant and consistent voluntary consumption of 10% alcohol over a period of 8 weeks. During the first 3 weeks of testing, alcohol rats consumed less sugar pellets and spent less time in reward boxes than controls. During the second 3 weeks, alcohol rats preferred constant over variable rewards and extinguished faster than controls. However, both groups displayed similar contrast effects for average pellets consumed during testing, indicating that voluntary alcohol consumption did not impact the ability to shift to optimal rewards. Our results suggest that alcohol consumption devalues reward outcomes, promotes preference for reliable rewards, and does not affect optimal reward-based decision making over time. Future work should further investigate how voluntary alcohol consumption may alter reward seeking, intake, and memory.

Disclosures: **J.J. McGraw:** None. **L.C. Zona:** None. **H.C. Cromwell:** None.

Poster

050. Alcohol Seeking, Reward, and Relapse

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 50.03/F18

Topic: C.17. Drugs of Abuse and Addiction

Title: Insular cortex and alcohol consumption: neurons encode reward expectancy during a operant conditioning task

Authors: ***S. VICENCIO**[1], M. AGUILAR[2], P. MALDONADO[1];
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Abstract: Drug dependent individuals present altered internal body states which leads to the increase of the drug's motivational effects, such as drug craving, thereby incrementing the risk of drug use. Because the insular cortex (IC), the highest structure in the interoceptive system, has been proposed as a region that integrates internal bodily states into decision-making processes involving rewards1, we examined the neuronal activity of IC in rats during an alcohol-craving paradigm.We designed an operant conditioning task that allowed us to perform single unit recordings at the IC while the rats were anticipating and also consuming a reward. Operant responding for ethanol was initiated by means of a sucrose solution substitution procedure. Afterwards, the animals were trained daily to respond for ethanol (10% w/v) or water under a two-lever free-choice paradigm. Then, animals learned to respond for a series of three light cues: the first indicated the trial start, the second showed which lever would be available (water or ethanol) and the third signaled the moment to start pressing the lever to obtain the reward. Over time, the rats developed a preference for ethanol over water and consumed enough ethanol to produce pharmacologically relevant blood alcohol concentrations. We recorded 120 single units from IC of three rats. We found that IC neurons display an increment in their firing rate when rats were expecting to receive ethanol and a decrease in the firing rate during the consumatory phase. These changes were no observed in omitted trials. These results suggests that the IC are directly involve in the neural representation of the emotional content of reward expectancy and also suggest that such changes in firing could reflect drug craving and its relief. References: 1. Naqvi NH, Bechara A (2009). The hidden island of addiction: the insula. Trends Neurosci Jan;32(1):56-67.

Disclosures: **S. Vicencio:** None. **M. Aguilar:** None. **P. Maldonado:** None.

Poster

050. Alcohol Seeking, Reward, and Relapse

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Title: Inactivation of CeA neuronal ensembles prevents alcohol drinking in dependent and non dependent rats

Authors: *G. DE GUGLIELMO[1], B. HOPE[2], O. GEORGE[1];
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[2]Neurobio. of Relapse Section, Natl. Inst. on Drug Abuse, Natl. Inst. of Hlth., Baltimore, MD

Abstract: Withdrawal from alcohol is associated with the recruitment of neurons in the central nucleus of amygdala (CeA) in rats binge-drinking on alcohol (non-dependent) as well as in dependent rats. However, whether the recruitment of this withdrawal neuronal ensemble in the CeA is causally related to excessive drinking or represents a consequence of the excessive drinking remains to be demonstrated. Here we tested the hypothesis that inactivation of the withdrawal neuronal ensemble in the CeA is responsible for the excessive alcohol drinking observed in non-dependent binge drinking rats and in dependent rats. In order to test our hypothesis we investigated the effect of the inactivation of neuronal ensembles in the CeA using a pharmacogenetic approach (Daun02 inactivation method in Fos-Lac Z transgenic rats). One group of rats was made dependent using chronic, intermittent exposure to alcohol vapor and tested for alcohol drinking in 30 minutes session 6-8 hours into withdrawal. In a second group of animals (non-dependent binge-drinker) we tested the effect of Daun02 on ethanol intake using chronic intermittent access to two-bottle choice. We found that inactivation of neuronal ensembles by injection of Daun02 in the CeA significantly decreased alcohol drinking in both groups with the only difference that in non-dependent animals the decreased ethanol intake was transient the day of the injection and returned to normal the day after the injection, while in the

dependent animals inactivation of the withdrawal neuronal ensemble produced a long-term decrease in alcohol drinking that lasted at least 2 weeks. We also found a significant reduction of the somatic withdrawal signs in the dependent animals injected with Daun02 in the CeA. These results demonstrate that the recruitment of a neuronal ensemble in the CeA during alcohol withdrawal is causally related to the excessive alcohol drinking observed in alcohol dependent rats but that this withdrawal neuronal ensemble only partially contributes to alcohol drinking in non-dependent rats.

Disclosures: G. De Guglielmo: None. **B. Hope:** None. **O. George:** None.

Poster

050. Alcohol Seeking, Reward, and Relapse

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 50.05/F20

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA10761

VA Medical Research

Title: The role of the ventral hippocampus in ethanol drinking and glutamate release in the nucleus accumbens

Authors: *W. C. GRIFFIN, III[1], H. L. HAUN[2], L. N. LUDERMAN[2], K. E. KOCH[2], M. KATES[2], H. A. BOGER[3], H. C. BECKER[2];
[2]Charleston Alcohol Res. Ctr, Psychiatry and Behavioral Sci., [3]Neurosciences, [1]Med. Univ. South Carolina, Charleston, SC

Abstract: Glutamatergic transmission within the nucleus accumbens (NAc) is important for regulating ethanol drinking and an important source of glutamate in the NAc is the ventral hippocampus (vHipp). The current study tested the hypothesis that the ventral hippocampus (vHipp) regulates ethanol drinking and contributes to glutamate release in the NAc. AAV containing inhibitory DREADDs under control of a synapsin promoter were infused into the vHipp. After 2 weeks of recovery, mice were habituated to injections and trained to drink ethanol in the binge-like drinking-in-the-dark paradigm. Systemic challenge (IP) with clozapine-N-oxide (CNO; 3 mg/kg) or vehicle (VEH; 0.9% saline) 30 min before ethanol access produced a 54% reduction in ethanol drinking versus the vehicle challenge (5.3 ± 0.7 g/kg vs. 2.9 ± 0.3 g/kg; $p < 0.05$). In a separate group of mice, following infusion of the inhibitory DREADDs into the

vHipp, *in vivo* electrochemistry procedures were performed to measure glutamate release in the NAc before and after CNO challenge. Mice where anesthetized using urethane (1.5 g/kg), placed in a stereotaxic apparatus and an enzyme-coated probe was lowered into the NAc. Once the probe stabilized (~1 hr), glutamate release was evoked by focal application of 70 mM KCl every 10 min, 4 times. Next, CNO (3 mg/kg) was injected and 30 min later glutamate release was measured again. Inactivating the vHipp to NAc projection neurons significantly reduced stimulated glutamate release ~40% (1.2 μM vs. 0.75 μM; $p < 0.05$). These data indicate that the vHipp to NAc projections play an important role in glutamate release in the NAc and, further, the vHipp contribute to the regulation ethanol drinking. Ongoing work is evaluating the influence of activating this pathway on ethanol drinking and glutamate release in the NAc.

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Poster

050. Alcohol Seeking, Reward, and Relapse

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Program#/Poster#: 50.06/F21

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA034389

Title: Evaluating the reward- enhancing effects of nicotine on ethanol self-administration in male and female rats

Authors: *S. BARRETT, S. LYON, S. PITTENGER, N. DUSZENKO, O. LOH, R. A. BEVINS;
Univ. of Nebraska - Lincoln, Lincoln, NE

Abstract: Nicotine and alcohol dependence disorders are highly correlated: up to 80% of alcohol-dependent persons in the US smoke regularly, and risk of alcohol-dependence is four times higher among people who are nicotine-dependent. Additionally, there are significant differences in the prevalence and nature of nicotine- and alcohol-dependence between the sexes. Women, on average, are less likely to attempt to quit smoking and more likely to relapse after quitting. Additionally, women show greater sensitivity to the intoxicating effects of alcohol, slower metabolism of alcohol, and greater risk for adverse health consequences alcohol use. However, binge drinking and alcohol-dependence is more common among men than women. Understanding the behavioral mechanisms that underlie alcohol- and nicotine-dependence and

their high comorbidity will require a consideration of the multifaceted causes that include an individual's sex. There is mounting evidence that the reward-enhancing properties of nicotine synergistically enhances behavior directed at obtaining other rewards, and this reward-enhancement effect holds promise in deciphering the mechanisms that drive nicotine dependence. That is, nicotine administration that occurs in the same context as the reception of other rewarding stimuli may enhance their rewarding properties, and this enhancement may drive motivation to abuse nicotine. However, no published work to date as investigated the role that reward-enhancement may play in the comorbidity between nicotine- and alcohol-abuse. The present work investigated the role that reward-enhancement by nicotine plays in the comorbidity of nicotine- and alcohol-abuse using an operant ethanol self-administration procedure in male and female rats. Rats were trained to self-administer 15% ethanol using a sucrose fading procedure. Nicotine or saline was injected 10 min preceding 45 min ethanol self-administration sessions. Over blocks of sessions, the unit cost per gram ethanol was systematically examined across two separate reinforcer demand assessment phases: fixed-ratio schedule escalation and ethanol concentration reduction. A behavioral economic, reinforcer demand model was applied to the data from both unit cost manipulation phases and the effects of nicotine on ethanol reinforcement value were compared between the sexes and between unit cost manipulation procedures.

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Poster

050. Alcohol Seeking, Reward, and Relapse

Location: Hall A

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Program#/Poster#: 50.07/F22

Topic: C.17. Drugs of Abuse and Addiction

Title: The effect of environmental enrichment on behavioral economic demand for ethanol and sucrose in Long-Evans rats

Authors: M. R. FARRELL, L. LANSBERRY, R. CAUGHRON, *M. P. MARTINETTI; Dept Psychol, The Col. of New Jersey, Ewing, NJ

Abstract: The literature on environmental enrichment (EE) and ethanol (EtOH) oral self-administration has revealed inconsistent results in animal models, with studies reporting that EE increases, decreases, or has no effect on EtOH consumption. Although these studies have addressed the effects of EE on EtOH preference, it is unclear whether EE affects the reinforcing

efficacy of EtOH. In a behavioral economic analysis, reinforcing efficacy can be defined as demand, or the extent to which EtOH consumption is "defended" when its price is increased. Demand analyses have been widely applied to the study of alcohol and drug self-administration in both human and animal models. Two key demand metrics include intensity of demand (i.e., consumption as price approaches zero) and sensitivity to price, or the degree to which consumption decreases when prices are increased. The current study assessed the effect of EE on behavioral economic demand for EtOH and sucrose in randomly bred rats. Sixteen male Long-Evans rats were randomly assigned to an EE group (housed with crinkle paper) or a control (Ctrl) group (no crinkle paper). All animals were trained to lever press for 10% EtOH (v/v) using a modified sucrose-substitution procedure. Thereafter, delivery of EtOH and concurrent 1% (w/v) sucrose solutions was contingent upon responding on concurrent fixed-ratio (FR) 4 schedules of reinforcement. After stable responding for EtOH and sucrose solutions was established, the FR value, or "price," of each of the solutions was independently increased (FR4, 8, 16, 32) every six sessions while the price of the alternative remained at FR4. In two control conditions, the price of each reinforcer was increased with water concurrently available on an FR4 schedule. The exponential demand equation (Hursh & Silberberg, 2008) was fitted to the behavioral economic demand data (mean reinforcer deliveries at each price) for each set of price manipulations and each group (EE and Ctrl). We found that rats in the EE condition demonstrated greater sensitivity to increases in EtOH and sucrose prices, across all conditions, compared with the Ctrl rats. In addition, EE rats demonstrated lower intensity of demand for sucrose compared with the Ctrl rats, but only when sucrose was concurrently available with water. These results suggest that EE reduces the reinforcing efficacy of both EtOH and sucrose as assessed in a behavioral economic paradigm.

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Poster

050. Alcohol Seeking, Reward, and Relapse

Location: Hall A

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Program#/Poster#: 50.08/F23

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA20539

Title: Efficacy of delta opioid receptor agonist induced β-Arrestin 2 recruitment positively correlates with alcohol intake in mice

Authors: *T. CHIANG[1], K. SANSUK[2], R. M. VAN RIJN[2];
[2]Medicinal chemistry and molecular pharmacology, [1]Purdue Univ., West Lafayette, IN

Abstract: The delta opioid receptor (DOR) is a member of the opioid receptor family that is known to be involved in many different physiological responses such as pain transmission, ischemic protection, depression and drug abuse. As suchTherefore DOR agonists are being developed as for therapeutic use, and some are already in clinical trials. However, no DOR selective agonist has currently received approval by the Federal Drug Administration nor has one even entered phase III clinical trials, which suggests perhaps the pharmacology is not fully understood. While most DOR agonists that have been studied are able to reduce depression-like behavior, we have previously shown that DOR agonists vary widely in their ability to modulate alcohol intake. More precisely some agonists decrease alcohol intake but other agonists can actually increase alcohol consumption. This would be a serious potential adverse effect for DOR agonist under development. This Sseemingly paradoxical behavior may be explained by the differences in receptor signaling bias. Here, we propose that DOR agonists that increase alcohol intake have different binding preference for β-arrestin 2 than those DOR agonists that decrease alcohol intake. To test our hypothesis we used two previously studied DOR agonists TAN-67 and SNC80, as well as four novel DOR agonists: two SNC80-like agonists (SNC162, AR-M390) and two agonists that do not structurally resemble SNC80 (KNT127, and NIH11082). We measured the ability of these agonists to inhibit cAMP and recruit β-arrestin 2 *in vitro* and we also determined how these agonist modulate alcohol intake in C57BL/6 mice using a voluntary, limited access 2-bottle choice paradigm. We found that there is a positive correlation between the efficacy of β-arrestin 2 recruitment by DOR agonists and alcohol intake in mice. More specifically, we found that 'super β-arrestin2 recruiters' increased alcohol intake and that super recruiters are dependent on β-arrestin 2 to function both with regard to alcohol behaviors and for their anti-depressant-like behaviors. These findings hold importance in future DOR agonist development for treatment for alcohol use disorders and depressive disorders.

Disclosures: T. Chiang: None. **K. Sansuk:** None. **R.M. van Rijn:** None.

Poster

050. Alcohol Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Title: Intravenous caffeine increases oral ethanol self-administration in rats

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Abstract: In recent years, caffeinated alcoholic beverages have risen in popularity. These combination beverages increase binge drinking, an effect that has been attributed to a reduction in the hypnotic (sleep-inducing) effects of alcohol. However, in our laboratory, we have shown that caffeine enhances the motivation to obtain non-drug rewards such as sucrose and saccharin (sweet tastes). Others have shown that caffeine can increase the reinforcing effects of cocaine. Since no previously published study has demonstrated that caffeine self-administered alone serves as a primary reinforcer (increases operant responding) we investigated whether caffeine infusions delivered in conjunction with oral alcohol could increase alcohol self-administration. Twenty male Sprague-Dawley rats were randomly assigned to one of three groups that received different combinations of oral and IV reinforcers (EtOH+SAL, EtOH+CAFF, or H2O+CAFF). EtOH denotes 20% (v/v) ethanol available orally, via a liquid dipper delivery system (0.1 mL); H2O denotes oral water; CAFF denotes IV infusions of caffeine, and SAL denotes isotonic saline infusions. Rats were initially shaped to respond for the oral reinforcer under a fixed ratio 2 (FR2) reinforcement schedule; after stable responding was observed they were instrumented for IV infusions. In subsequent tests, the assigned infusion (CAFF or SAL) was delivered each time a rat earned an oral reinforcer (EtOH or H2O). Caffeine infusions (0.05-0.4 mg/kg/ing) increased operant responding for alcohol in the EtOH+CAFF group, relative to the EtOH+SAL group in a dose dependent manner. Caffeine dose was inversely related to operant responding, with the lowest dose tested (0.05 mg/kg/inf) producing the most robust increases in responding. Rats were subsequently shifted to a progressive ratio (PR) reinforcement schedule which measures motivation by increasing the amount of effort required to earn each reinforcer. The EtOH+CAFF group reached a significantly higher breaking point than the other two groups at the 0.1 mg/kg/inf dose. These findings implicate the reward-enhancing effects of caffeine in the binge-intoxication associated with caffeinated alcoholic beverages.

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Poster

050. Alcohol Seeking, Reward, and Relapse

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VA Medical Research

Title: Oxytocin reduces binge-like alcohol drinking

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Abstract: Accumulating evidence demonstrates a role for the neuropeptide oxytocin (OXT) in modulating drug-taking behavior. Specifically, studies have shown that administration of OXT decreases alcohol (ethanol), opiate, and methamphetamine self-administration, and prevents the development of tolerance and withdrawal symptoms to these drugs. The present study was designed to extend these findings by examining the effects of OXT on binge-like ethanol consumption using the drinking-in-the-dark (DID) model.. Adult male C57BL/6J mice were presented in the home cage with a single bottle of 20% (v/v) ethanol in place of water starting 3 hours into the dark cycle. In this 4-day paradigm, the ethanol access period was for 2 hr/day for three consecutive days and extended to 4 hr/day on the final (fourth) day. Vehicle injections (IP) were given 30 min prior to each of the first three drinking sessions in order to acclimate mice to the injection. On Day-4, mice were administered OXT (0, 0.3, 1, 3, or 10 mg/kg) 30 min prior to the test session. Results indicate that OXT reduced ethanol intake in a dose-related manner, with maximal reduction (~50% reduction) produced by 3 and 10 mg/kg OXT doses ($p < 0.001$). Separate studies using lickometer circuitry to track temporal patterns of drinking indicated that OXT produced a significant delay in onset of drinking and assessment of open-field locomotor activity indicated that the reduction in drinking is not simply due to impaired locomotion. Next, we examined whether blockade of the oxytocin receptor antagonized oxytocin's effects on ethanol consumption. Prior to drinking, mice were administered a selective oxytocin receptor antagonist L368,899 (0,1,3, 10 mg/kg) followed by injection of OXT (1 mg/kg) or saline. Results indicated that the oxytocin-induced reduction in drinking was blocked by the 10 mg/kg dose of the oxytocin receptor antagonist L368,889. Taken together, these data suggest a role for oxytocin, mediated through the oxytocin receptor, in binge-like ethanol consumption. Studies are underway to investigate the potential role of vasopressin (V1b) receptor blockade in mediating oxytocin's effect on binge-like ethanol consumption.

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Poster

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U01AA016647

Title: Effects of ghrelin antagonist D-Lys3-GHRP-6 on alcohol consumption may be mediated by the Urocortin 1 peptide

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Abstract: Heavy alcohol consumption has many deleterious effects on behavior and physiology and animal models such as mice are useful to test mechanisms regulating alcohol intake and dependence. These studies have led to novel theories on mechanisms that attribute to alcohol dependence and possible treatments using behavioral and pharmacological strategies. A number of recent studies have pegged the ghrelin system as a contributor of alcohol drinking in both human and rodent models, and ghrelin antagonists are a promising treatment to decrease alcohol abuse. Similarly, Urocortin 1 (UCN1), a peptide of the corticotropin releasing family, has been implicated in regulation of alcohol drinking in mouse studies. UCN1 is preferentially expressed in the centrally-projecting Edinger-Westphal nucleus of the brain. This nucleus also expresses high levels of ghrelin receptors. It is possible, therefore, that UCN1 and ghrelin regulate alcohol consumption through overlapping mechanisms. In the current experiment we used female wild-type (WT) and knockout (KO) UCN1 mice to examine the effects of a ghrelin receptor antagonist D-Lys3-GHRP-6 (DLys) on alcohol drinking over 7 days. Following baseline acquisition of alcohol intake mice were injected daily with DLys (15 mg/kg) beginning at lights off and presented with 10% v/v ethanol in a 24-hour two-bottle choice procedure. Alcohol, preference, water, and food intake was measured 4- and 24-hours post-injection. At the onset of drug administration, DLys reduced alcohol intake in wild-type, but not KO mice. This decrease diminished following 2-3 days of administration. The reduction in alcohol intake was accompanied by a reduced preference for alcohol in the DLys group, but again this was only present in WT mice, not KO. Generally, DLys increased water intake, but only 4-hours post-injection, and had no effect on food intake. The effects seen in the female WT mice are similar to those previously found in our lab in male C57BL/6J mice. However, in the current study DLys-

induced reduction of alcohol intake was still present at 24-hours, a result not seen in males. Given the effects of DLys were present in WT, but not KO mice, we hypothesize that ghrelin (in part) works through the UCN1 system to mediate alcohol intake in female mice. Future studies need to further investigate interactions of between the UCN1 and ghrelin systems.

Disclosures: J.L. Gomez: None. **A.E. Ryabinin:** None.

Poster

050. Alcohol Seeking, Reward, and Relapse

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Title: Ketamine reduces alcohol intake in olfactory bulbectomized rats

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Abstract: The co-morbid condition of depression and alcoholism is a well-established epidemiological finding. Although the exact cause-effect of this phenomenon is not at hand, it is known that high consumption of alcohol can lead to numerous neurochemical alterations that can precipitate depressive-like behavior. On the other hand, initial low or moderate alcohol intake may result in euphoric or dissociative feelings and hence may be used to counteract the low mood. However, regardless of this initial effect, such self-medication by alcohol may lead to rapid tolerance and eventual exacerbation of the depressive characteristics. Indeed, mood dysregulation following abrupt alcohol withdrawal may be a common cause for relapse to alcoholism. Ketamine, an NMDA receptor antagonist with potent dissociative anesthetic properties has been shown to have rapid and lasting antidepressant effects. The two major goals of this study were: 1. To determine whether olfactory bulbectomized rats, a putative animal model of depression, will consume more alcohol, and 2. If so, whether an acute administration of ketamine to such animals would reduce their alcohol intake. The results indicate that bulbectomized (OBX) adult male Wistar rats consumed significantly more alcohol during the entire 3-week period compared to the sham-operated controls. Moreover, intraperitoneal administration of a low ketamine dose of 5 mg/kg 20 min prior to the alcohol session dramatically reduced the amount of alcohol consumed by these OBX animals in terms of actual

amount and % of baseline intake. Interestingly, the same ketamine dose also reduced the % of baseline alcohol intake in sham-operated animals. These results indicate that depressive mood can lead to higher alcohol consumption and that a novel antidepressant such as ketamine could mitigate alcohol intake in such depressive-like conditions. However, since ketamine also had an effect on alcohol intake in sham-operated animals, it remains to be determined whether the effect of ketamine on alcohol consumption is solely due to its antidepressant properties, or may also arise from some other pharmacodynamic interactions with alcohol. Supported by: NIH/NIAAA R03AA022479 (YT), project "CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund and project of specific research at the Masaryk University (MUNI/A/1116/2014) (JK, ZB).

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH AA022448

Title: Moxidectin alters dopamine levels and reduces ethanol intake in female mice: implications for developing novel therapeutic agents for alcohol use disorder

Authors: N. HUYNH[1], N. ARABIAN[1], J. THUY[1], H. PEI[1], L. ASATRYAN[1], S. LOUIE[1], M. JAKOWEC[2], *D. L. DAVIES[1];
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Abstract: Considerable efforts have been put forth on drug development for alcohol use disorder (AUD) which has yielded limited success. Currently, there are only three FDA-approved medications for the treatment of AUD. Unfortunately, high rates of uncontrolled heavy drinking continue to persist highlighting the needs for the development of new and effective medications for AUD. Our approach has focused on repurposing ivermectin (IVM), an FDA-approved drug for the treatment of onchocerciasis, as a novel pharmacotherapy for AUD. Preclinically, IVM significantly reduces acute and longer term ethanol intake in mice. Despite promise, IVM's weak blood brain (BBB) penetration may limit its utility as a long-term treatment for AUD. As such, we have started testing another avermectin, moxidectin (MOX) for use against AUD. MOX has superior BBB penetration compared to IVM and may represent another pharmacotherapy for

AUD. The present study tested the effects of MOX administered for 5 days using a 24-h-two-bottle choice paradigm, where mice had continuous access to both 10% ethanol v/v solution and water, and also using a Drinking-in-the-Dark paradigm, where mice had 2 hours access to 20% ethanol v/v solution during the dark phase resulting in consumption of high levels of ethanol similar to what is reached in humans while binge drinking. In both paradigms, we found that 2.5 mg/kg MOX significantly reduced ethanol intake in female C75BL/6 mice across the five days. Importantly, the mice exhibited no signs of overt toxicities. In addition, changes in dopamine levels were identified using HPLC method at the end of day one and day five in the prefrontal cortex and nucleus accumbens for MOX treated mice vs. saline controls suggesting the ability of MOX to modulate mesolimbic dopamine activity. These findings, coupled with our previous work on IVM, suggest that avermectins may represent a new class of compounds that can be developed as novel therapeutics for AUD. Support: AA022448 (DLD) and the USC School of Pharmacy.

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Poster

050. Alcohol Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Title: Mechanical stimulation of the HT7 acupuncture point reduces ethanol self-administration in rats

Authors: *S.-Y. KANG, Y. RYU, O. KWON, S. YEON, S. CHO, K.-H. CHOI, J. KIM, M. KIM, Y. SHIN, J.-W. CHOI;
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Abstract: Background: Alcoholism, a disabling addiction disorder, is a major public health problem in the U.S. and worldwide. Acupuncture has been shown to alleviate various drugs of abuse, including alcohol. The present study was designed to determine whether the application of acupuncture at Shenmen (HT7) points suppress voluntary alcohol consumption in the rats and whether this suppressive effect is potentiated by administration of naltrexone, the clinically approved medication for alcoholism in combination with acupuncture. Methods: Rats were initially trained to self-administer a sucrose solution (20% w⁄v) to facilitate operating lever. After stable baseline responding was established, the sucrose concentrations were gradually

decreased to 0% and ethanol concentrations were increased to 10%. After rats showed stable responding for 10% ethanol and met an established criterion for ethanol baseline responding, behavioral testing was initiated. A mechanical acupuncture instrument (MAI) for objective mechanical stimulation was given to rats whose baseline responding had been determined prior to the test session. Additionally, the effects of naltrexone (0.1, 0.3, 1, 3mg／kg) on ethanol self-administration were investigated in different groups of rats. Results: We found that the ethanol intake in rats under the application of MAI at HT7, but not Joksamli (ST36) and tail point were significantly reduced. Whereas the treatment of naltrexone at high dose (1, 3mg/kg) significantly reduced ethanol intake, low dose of naltrexone (0.1, 0.3mg/kg) did not produce any reducing effect. Especially, low-dose injection of naltrexone in conjunction with MAI synergistically suppressed ethanol intake. Conclusions: The results of current study indicate that MAI at HT7 effectively reduces ethanol consumption in rats. Furthermore, co-administration of MAI and low-dose naltrexone can produce more potent reducing effect of ethanol intake than one treatment, indicating a potential novel strategy for the management of alcoholism.

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Poster

050. Alcohol Seeking, Reward, and Relapse

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Topic: C.17. Drugs of Abuse and Addiction

Title: The effects of brief conditioning trial duration on ethanol-induced conditioned place preference in mice

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Abstract: Previous studies have demonstrated that the timing of ethanol administration relative to cue exposure (CS) can affect the outcome of place conditioning in mice. If ethanol is administered before exposure to a floor CS, a preference is developed, whereas if ethanol is administered after or during exposure to the floor CS, an aversion is developed (Cunningham et al., 1997, 2000, 2003). To explain these findings, Cunningham has proposed a biphasic motivational response whereby ethanol administration results in a short-lived aversive effect followed by an enduring rewarding effect. In an attempt to measure this brief aversive phase, we examined the effects of a brief conditioning trial duration on conditioned place preference. A

place conditioning procedure was used in which adult male DBA/2J mice were exposed to 4 pairings of 2 g/kg ethanol with a distinctive floor stimulus. Saline was administered on alternate days and paired with a different floor. Conditioning trials lasted 1 or 5 minutes and the experiment concluded with four 60-minute floor preference tests 1, 3, 8, and 10 days after the final conditioning day. Contrary to what would be predicted if ethanol injection has an initial, aversive motivational effect, mice in the 1 min trial group demonstrated conditioned place preference after 4 trials, as did mice receiving our standard 5 min trials. Additionally, we found no difference in the strength of learning between groups; there was no difference in magnitude of preference and preference was extinguished at approximately the same rate in 1-min and 5-min trial groups over multiple drug-free preference tests. Thus, these findings suggest that the rewarding effects of ethanol develop within the first minute after injection and do not support the biphasic motivational response theory. Strikingly, four 1-minute pairings of 2 g/kg ethanol with a floor cue produced a preference that persisted through more than two hours of drug-free preference tests. Further research is needed to explain why ethanol administration after cue exposure results in a conditioned place aversion.

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Poster

050. Alcohol Seeking, Reward, and Relapse

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KK1512-02

Title: Potential roles of the nucleus accumbens and amygdala mGluR5 in the acquisition and expression of ethanol-induced place preference

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Abstract: We have previously demonstrated that the type 5 metabotropic receptor (mGluR5) attenuated the rewarding effects of ethanol. Here we investigated functional roles of mGluR5 in the nucleus accumbens and the amygdala on the acquisition or expression of ethanol conditioned place preference in mice. In the first set of experiment, mice were intraperitoneally conditioned

with saline or ethanol (20% v/v, 1.5 g/kg) on alternating days for 8 consecutive days and received local injection of the mGluR5 antagonist MPEP into the nucleus accumbens or the amygdala immediately before ethanol conditioning. Result showed that 1.5 g/kg ethanol alone did not induced ethanol place preference, whereas inactivation of mGluR5 receptors produced by MPEP enhanced conditioned rewarding effects of ethanol. These results indicate that activation of mGluR5 plays a significant role in inducing conditioning rewarding effects of ethanol. In the second set of experiment, mice were conditioned with saline or ethanol (20% v/v, 2.0 g/kg). After ethanol conditioning, MPEP was administered into the nucleus accumbens or the amygdala prior to the post-conditioning. Results showed that pretreatment with MPEP reduced the expression of ethanol-induced conditioned place preference. Taken together, these data suggest that pharmacologically blockade of the mGluR5 in the nucleus accumbens and the amygdala differentially affect the acquisition and expression in the condition place preference of ethanol. *Supported by a grant (14182MFDS979) from Ministry of Food and Drug Safety in 2015 and Korea Institute of Toxicology (KK1512-02).*

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Poster

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Title: Role of projections from dorsal medial prefrontal cortex to nucleus accumbens core in context-induced reinstatement of ethanol seeking

Authors: *P. C. BIANCHI[1], R. M. LEÃO[1], P. E. CARNEIRO-DE-OLIVEIRA[1], F. C. CRUZ[2], C. PLANETA[1];
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Abstract: In human addicts, relapse is often precipitated by re-exposure to environmental contexts that were previously associated with drug use. Specific patterns of sparsely distributed neurons, called neuronal ensembles, have been hypothesized to encode learned associations

between drug-associated contexts and drug effects. Male and Female Long Evans rats were trained to self-administer ethanol 10% (1h/day for 14 days). Drug infusions were paired with a discrete tone-light cue. Subsequently, lever responding was extinguished over 10 days in the presence of the discrete cue in a non-ethanol context with different sensory features than the drug self-administration context. Rats were then re-exposed to the ethanol-associated context (or the non-drug extinction context as the control condition) and lever pressing was assessed under the same extinction conditions for 60 min as a measure of ethanol seeking. Neuronal ensembles in dorsal medial prefrontal cortex that were activated during context-induced reinstatement were identified using Fos immunohistochemistry. We also determined the proportion of dorsal medium prefrontal cortex (dmPFC) neurons expressing Fos during the reinstatement test by double-labeling Fos and the neuron-specific protein marker (NeuN). Reexposure to the ethanol-associated context reinstated alcohol seeking (active lever presses: 16.61 ± 2.59, extinction context and 34.78 ± 6.32, training context; n=14 per group) and increased expression of the neural activity marker Fos in the dorsal medial prefrontal cortex (fos/mm2 - extinction context: 38,9 ± 6,6 n=6 and training context: 60,6 ± 9,3; n=5). Double-labeling for Fos and NeuN indicated that in the dmPFC, only a small proportion of neurons were activated during context-induced ethanol seeking (5,6 ± 0.86% in the extinction context and 6,4 ± 0.4% in the training context). Conclusions: Our results showed context-induced alcohol seeking correlated with activation of dmPFC. Future experiments: We will analyze by double-labeling for Fos and NeuN immunohistochemical detection the neural activation in the accumbens (Acc) core. Furthermore, we will determined neuronal activation in Acc core brain area projecting to dmCPF during etanol context-induced tests by measuring double labeling of the retrograde tracer cholera toxin subunit B (CTb; injected in Acc core) with Fos.

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Poster

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Title: Decreased neurogranin in the nucleus accumbens promotes the reward motivation and reinforcement for ethanol seeking

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Abstract: Striatal glutamate signaling has been implicated in the pathophysiology of neurological disorders and alcoholism. NMDA glutamate receptors play an essential role in both reward motivation and seeking behaviors, but its intracellular target is not well studied. Neurogranin (Ng) is predominantly expressed on the postsynaptic dendrite and is an intracellular target of NMDAR signaling, however, evidence for the role of Ng signaling in alcohol related behaviors have not yet been established. In the present study, we identified that the dampening of NMDAR-Ng signaling enhances the reward motivation during Pavlovian conditioning and promotes excessive ethanol seeking during operant conditioning, while also increasing sensitivity to ethanol intoxication. We found that the lack of Ng in the nucleus accumbens (NAc) increases intrinsic motivation and insensitivity to aversive effects of reinforcement during ethanol seeking behaviors. Ng -/- mice also show enhanced motivation measured by a progressive ratio schedule (PR) schedule, while Ng +/+ mice showed significant increased response by NAc specific optogenetic stimulation. Finally, we demonstrated that chronic ethanol administration could reduce both the length of axons and mGluR5 expression in the NAc, which promotes excessive ethanol drinking in Ng -/- mice. Our results indicated that Ng is a key determinant in the postsynaptic intracellular mechanism resulting from NMDAR and is associated with a higher susceptibility of enhanced reward motivation for excessive ethanol drinking.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA 2P50AA012870

Title: Target-specific ablation of medial prefrontal cortex projections in extinction and reinstatement of alcohol-seeking behavior

Authors: *C. KEISTLER[1], J. M. BARKER[4], E. M. HAMMARLUND[1], C. W. BOND[2], M. XU[3], C. PITTENGER[3], R. J. DILEONE[3], J. R. TAYLOR[3];
[2]Interdepartmental Neurosci. Program, [3]Psychiatry, [1]Yale Univ., New Haven, CT; [4]Med. Univ. of South Carolina, Charleston, SC

Abstract: Alcohol is one of the most widely abused drugs in the United States and relapse presents a considerable challenge for recovering alcoholics. Relapse is often driven by alcohol-associated cues that trigger craving even after extended abstinence. Reducing the probability of relapse in alcoholics will require a better understanding of the neurobiology that mediates these behaviors. Previous research has focused on the regulation of alcohol seeking by limbic-striatal circuitries - especially basolateral amygdala (BLA) and nucleus accumbens shell (NAcS). However, the functional regulation of these circuits by the medial prefrontal cortex (PFC) has received little investigation. We previously used a contralateral lesion strategy to functionally disconnect infralimbic PFC (ilPFC) from NAcS and BLA to assess the necessity of these pathways for alcohol-related behaviors. We found that disconnection of ilPFC and NAcS increased alcohol self-administration and cue-induced reinstatement, whereas disconnection of ilPFC and BLA decreased these behaviors. However, a limitation of these experiments is that lesions disrupt the circuit in both directions. A remaining question is whether "top-down" neurons projecting from ilPFC to each structure serve a different role than "bottom-up" neurons projecting in the opposite direction. Here we demonstrate the utility of a novel technique for more specific disruption of neural circuits based on their projection targets. Specifically, we inject a Cre-dependent virus expressing diphtheria toxin receptor (DTR) into ilPFC and a retrogradely transported AAV6-Cre virus into NAcS or BLA. This leads to expression of DTR only in ilPFC neurons projecting to the selected subcortical target, and enables specific ablation of these neurons by systemic injection of diphtheria toxin (DT) at any subsequent time point. In the current experiments, we inject DT after animals learned to self-administer alcohol, then test the necessity of ilPFC → NAcS projection neurons for extinction and cue-induced reinstatement of alcohol seeking. Preliminary results suggest that ablation of ilPFC → NAcS neurons reduces cue-induced reinstatement of alcohol seeking but does not affect extinction. Given that ilPFC also connects reciprocally with BLA, current experiments are testing the separate effects of either top-down ilPFC → BLA or bottom-up BLA → ilPFC ablation on these same relapse-associated behaviors.

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Poster

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DA026356

Title: Interactive effects of ethanol and HIV-1 viral proteins on novelty-seeking behaviors

Authors: T. WINGO[1], T. NESIL[1], S. CHANG[2,3], *M. D. LI[1];
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Abstract: Novelty-seeking behavior is related to the reward system in the brain and could predict the potential for addiction. Alcohol use is prevalent in HIV-1 infected patients and adversely affects anti-retroviral medication. The difference in vulnerability to alcohol addiction between HIV-1 infected and non-infected populations has not been fully investigated. This study was designed to determine whether HIV-1 viral proteins alter the effects of ethanol on novelty-seeking behaviors in the HIV-1 transgenic (HIV-1Tg) rat. The hole board and open field tests were used to first compare the baseline (pre-session ethanol) novelty-seeking behaviors in HIV-1Tg and F344 control rats. Rats then received a single daily intra-peritoneal injection of ethanol (1 g/kg) or saline for 13 d. The rats were then re-exposed to the hole board and open field test on the Days 10 and 11 (post-session ethanol trials) to determine the combined effects of HIV-1 viral proteins and chronic ethanol exposure on novelty-seeking behaviors. There was a significant strain difference in baseline novelty-seeking behaviors; HIV-1Tg rats exhibited both higher head dip scores in the hole board test ($p = 0.001$) and center entry scores ($p = 0.01$) in the open field test compared to the F344 control rats. In the post-session ethanol trials, novelty-seeking behaviors were increased in the ethanol-treated groups, but decreased in the saline control groups in both the hole board ($p = 0.001$) and open field ($p = 0.004$) tests. Ethanol-treated HIV-1Tg rats had higher head dip ($p < 0.01$) and center entry ($p < 0.05$) scores compared to the ethanol-treated F344 rats. There was a significant difference in head dip ($p = 0.03$) and center entry ($p = 0.02$) scores in pre- and post-ethanol treatment sessions, respectively, in the HIV-1Tg rats. Our results indicate that HIV-1 viral proteins alter novelty-seeking behaviors, induce neuronal adaptations in the mesolimbic reward pathway, and enhance the effects of ethanol on novelty-seeking behaviors. Using the novelty-seeking trait as a correlative behavioral marker of alcohol dependence could have important implications for the development of new psychopharmacological treatments for alcohol-dependent HIV-1 infected patients.

Disclosures: **T. Wingo:** None. **T. Nesil:** None. **S. Chang:** None. **M.D. Li:** None.

Poster

050. Alcohol Seeking, Reward, and Relapse

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 50.21/F36

Topic: F.01. Human Cognition and Behavior

Support: AA023165

AA010723

AA012388

AA017168

AA017923

Title: Brain activation to cannabis- and alcohol-related words in alcoholism

Authors: ***T. SCHULTE**[1,2], A.-P. LE BERRE[3], M. SERVENTI[3], J. METZLER[2], E. V. SULLIVAN[3], A. PFEFFERBAUM[1], E. M. MÜLLER-OEHRING[3];
[1]Neurosci Program Ctr. Hlth. Sci., SRI Intl., Menlo Park, CA; [2]Palo Alto Univ., Pacific Graduate School of Psychology, CA; [3]Psychiarty and Beh. Sci., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Cannabis abuse commonly occurs with alcohol use disorder. With increased acceptance and accessibility to cannabis in the US, it is imperative to understand the potential consequences on cognition and behavior. Taking a lead from alcohol research, alcohol-related stimuli can become conditioned cues with repeated alcohol consumption by associating the stimulus with the rewarding effects of alcohol. In alcoholism, an implicit attentional bias toward alcohol-related stimuli has been associated with craving and relapse and at the neural level with activation of midbrain-based cortico-striatal and limbic brain systems such that processing of these stimuli takes precedence over cortical control systems. We hypothesized that cue-sensitive reward systems may be also responsive to other drug-related stimuli. Here we used fMRI to examine the neural correlates of alcohol- and cannabis-related stimuli in 11 abstinent alcoholics (ALC) (abstinent from alcohol median=25 weeks; n=6 with past cannabis abuse, abstinent median=26 years) and 10 age-matched healthy controls. The task was to match the color of a patch to the font color of a word by pressing a Yes-key for color matches and a No-key for non-

matches. Words were congruent color words (e.g., the word RED in red font), alcohol-related (WINE, BEER), or cannabis-related words (WEED, DOPE) written in green, red, or blue font. As expected, participants showed faster response times for color matches than non-matches. Groups did not differ in overall task performance (response time (RT), errors, misses). An interaction between group and task conditions revealed that relative to controls, ALC had faster RT to cannabis than alcohol words and were slowest to color words. Shorter alcohol abstinence in ALC correlated with faster RT to alcohol (vs. color) words (Rho=.67). ALC deactivated premotor and cingulate cortices to alcohol (vs. color) words, and the extent of the deactivation was related to shorter alcohol abstinence (Rho=.66), whereas controls activated midline frontoparietal cortical areas. For cannabis (vs. color) words, ALC engaged midbrain, hippocampal, and cerebellar regions more than controls, whereas controls activated medial frontal, premotor, and cingulate regions more than ALC. Greater cerebellar activity in ALC correlated with greater response facilitation to cannabis words (Rho=-.73). These results suggest that both cannabis and alcohol stimuli in alcohol dependence can activate midbrain-limbic and cerebellar systems associated with stimulus-driven attention and response facilitation. Support: AA023165, AA010723, AA017923, AA012388, AA017168

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Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

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Program#/Poster#: 51.01/F37

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01DA033479-02

NIH Grant P50DA015369

Title: Cocaine-induced alterations in structural plasticity in the dmPFC of rats during early withdrawal

Authors: ***B. M. SIEMSEN**, P. MULHOLLAND, J. KOERBER, S. LANDER, P. KALIVAS, J. MCGINTY;
Med. Univ. of South Carolina, Charleston, SC

Abstract: Relapse to drug-seeking is a major clinical obstacle in treating addiction. Numerous studies have shown that dysregulation of the dorsomedial prefrontal cortex (dmPFC) to nucleus accumbens core (NAc core) glutamatergic projection precipitates relapse in rodent models. Altered synaptic plasticity and dendritic spine morphology and density (structural plasticity) within corticolimbic circuitry are associated with different phases of withdrawal and relapse. Previously, our lab has shown that cocaine self-administration (SA) in rats results in a depression of ERK, CREB, and NMDA receptor subunits, GluN2A and GluN2B, phosphorylation. These signaling alterations are associated with a parallel increase in striatal-enriched tyrosine phosphatase (STEP) activity in the dmPFC two hours after the final of 14 short access SA sessions (early withdrawal), suggesting decreased synaptic activity. A similar decrease in ERK and CREB phosphorylation, as well as GluN2A/B expression, is associated with decreased apical dendritic complexity in stress and depression models. In light of these observations, the current study investigated whether cocaine SA decreases apical spine head diameter (dH) and density in the dmPFC during early withdrawal. Fifteen male Sprague Dawley rats self-administered cocaine or were exposed to non-contingent yoked saline infusions for 14 days, and were transcardially perfused two hours after the final session. Coronal sections containing the dmPFC were labeled with the lipophilic dye DiI, and apical dendrites were acquired with confocal microscopy, and analyzed with Imaris 3D image analysis software. Results indicate that cocaine SA decreases layer II/III apical spine density as well as layer V apical spine dH ($p < 0.05$). The decreased layer II/III apical spine density is specific to a loss of thin and mushroom type spines. Ongoing experiments are delineating whether these alterations are occurring in dmPFC neurons that project to the NAc core using retrogradely transported fluorescent microspheres injected into the NAc core. We predict this alteration in structural plasticity plays a role in decreased synaptic activity during early withdrawal. This alteration may be facilitating relapse following abstinence because a single BDNF microinfusion immediately after SA suppresses relapse by reversing a cocaine-induced depression of phosphoproteins in the dmPFC during early withdrawal. Thus, investigation as to whether a BDNF microinfusion, or STEP inhibition, will reverse these cocaine-induced alterations in structural plasticity is a future direction.

Disclosures: **B.M. Siemsen:** None. **P. Mulholland:** None. **J. Koerber:** None. **S. Lander:** None. **P. Kalivas:** None. **J. McGinty:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 51.02/F38

Topic: C.17. Drugs of Abuse and Addiction

Support: DA033479

Title: Alterations in dmPFC neuronal activity during and immediately after cocaine self-administration

Authors: *T. S. DENNIS, J. A. KOERBER, T. C. JHOU, J. F. MCGINTY;
Med. Univ. of South Carolina, Charleston, SC

Abstract: Previous work from our laboratory has identified a down-regulation of activity-related phospho-proteins (p-GluN2A/B, pERK and pCREB) in the dorsomedial PFC (dmPFC) during early withdrawal from cocaine self-administration (SA) that appears to be crucial for relapse. Reversing these changes with a single infusion of brain-derived neurotrophic factor (BDNF) into the dmPFC immediately after the last cocaine SA session suppresses subsequent cue- and cocaine-induced drug-seeking in a TrkB- and ERK-dependent manner, and normalizes cocaine-induced dysregulation of glutamate levels in the nucleus accumbens (NAc) that are associated with relapse. Given the profound therapeutic potential of this understudied time point, the current study aims to explore direct measurements of neuronal activity in the dmPFC during SA and early withdrawal through the use of *in vivo* single-unit electrophysiological recordings in awake, behaving rats. Male Sprague Dawley rats were implanted with an intra-jugular catheter and a drivable 16-wire electrode bundle into the dmPFC and allowed to recover for 5 days. Rats were habituated to the SA chamber for 1 day before taking a 4 hr and 50 min baseline recording to assess the activity of dmPFC neurons in the absence of any stimulation. Rats were then trained to self-administer cocaine on an FR1 schedule, with electrophysiological recordings taken on Days 1-2 (Early SA) and Days 11-12 (Late SA). Neural activity was continually recorded during a 20 min baseline, during the 2 hr cocaine SA period, and during the 2.5 hr post-cocaine SA period. Preliminary data suggest that cocaine SA in cocaine-experienced (but not naïve) animals suppresses neuronal firing in the dmPFC when compared to the 20 min baseline. Interestingly, after the levers are withdrawn and cocaine access is taken away (indicating the beginning of early withdrawal), neuronal activity increases dramatically and is sustained at a higher rate of firing through the end of the session. This increase in neuronal firing during early withdrawal may paradoxically explain the decreases in NMDA receptor-related phospho-proteins that are observed 2 hr after the final SA session. It is likely that increases in neuronal activity following cocaine-induced suppression are robust enough to induce calcineurin-dependent phosphatases that dephosphorylate GluN2A/B receptor subtypes and ERK. These data show promise in expanding our understanding of the mechanisms involved during cocaine SA and in early withdrawal. Supported by DA033479

Disclosures: **T.S. Dennis:** None. **J.A. Koerber:** None. **T.C. Jhou:** None. **J.F. McGinty:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH P50 DA15369

NIH T32 DA007288

Title: Inhibition of Src family kinases prevents the suppressive effect of BDNF on cocaine-seeking

Authors: *S. M. BARRY, E. L. HERZIG, J. F. MCGINTY;
Neurosci. Inst., Med. Univ. of South Carolina, Charleston, SC

Abstract: Relapse to drug seeking remains a major obstacle in the treatment of cocaine addiction in human addicts. Animal models of relapse have demonstrated that neuroadaptations in reward circuits following cocaine self-administration underlie reinstatement to drug seeking. Specifically, dysregulation of the pathway from the prefrontal cortex (PFC) to the nucleus accumbens (NAc) is implicated in reinstatement. Brain-derived neurotrophic factor (BDNF) is synthesized in PFC pyramidal neurons and anterogradely transported to the NAc where it is the primary source of BDNF. Our lab has shown that a single BDNF infusion into the prelimbic cortex following a final cocaine self-administration session results in attenuation of reinstatement to cocaine-seeking. Inhibiting BDNF's receptor, TrkB, ERK/MAP kinase activation, or AMPA/NMDA receptors can block this attenuating effect. These results imply that the interaction between glutamate-mediated synaptic activity and TrkB signaling is imperative to BDNF's suppressive effect on drug-seeking. Src family kinases (SFKs) are involved in both NMDA/AMPA-mediated activation of TrkB and TrkB-mediated phosphorylation of NMDA receptors. Thus SFKs serve as a likely link between these two signaling systems. We hypothesized that infusion of the SFK inhibitor, PP2, into the prelimbic cortex prior to a BDNF infusion immediately after the end of the last cocaine self-administration session will block BDNF's attenuation of both context- and cue-induced reinstatement in rats. PP2 blocked BDNF's suppressive effect on context-induced relapse after one week of abstinence and cue-induced reinstatement after extinction. Because cocaine induces a dephosphorylation of GluN2A and GluN2B receptors and BDNF reverses this action, PP2 is likely blocking this reversal. Analysis of phospho-GluN2A/B, phospho-ERK, and activated SFK levels performed on the automated immunoassay system WES^{TM} (Protein Simple, BioTechne) will be presented to determine if PP2's blocking action occurs from dysregulation of TrkB-mediated NMDA receptor activation.

Disclosures: **S.M. Barry:** None. **E.L. Herzig:** None. **J.F. McGinty:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 51.04/F40

Topic: C.17. Drugs of Abuse and Addiction

Support: R01DA034806

Title: Involvement of CaMKII within the prefrontal cortex and the nucleus accumbens in the effects of Taar1 agonist on reinstatement of cocaine seeking in rats

Authors: ***J. LIU**, J.-X. LI;
Pharmacol. and Toxicology, Univ. at Buffalo, SUNY, Buffalo, NY

Abstract: The trace amine-associated receptor 1 (TAAR1), a novel G protein coupled receptor, has proven to play a crucial role in modulating the dopaminergic system in brain. Our recent study demonstrated that systemic administration of TAAR1 agonist decreased cue- or drug-induced reinstatement of cocaine seeking in rats. However, the neuronal mechanism underlying the role of TAAR1 in cocaine addiction remains unknown. Here, we examined the effect of selective TAAR1 agonist RO5166017 on cocaine seeking behavior in rats, and investigated the underlying mechanism. Rats were tested drug-induced reinstatement one day after extinction of cocaine self-administration. Immediately after cocaine reinstatement test, rats were decapitated and tissues of two critical brain regions in drug addiction, the prefrontal cortex (PFC) and the nucleus accumbens (NAc), were harvested for examining the alterations of related molecules. The results showed that pretreatment of RO5166017 (10 mg/ kg, i.p.) reduced cocaine priming-induced reinstatement of cocaine seeking. After reinstatement, the levels of phosphorylated Ca2+/calmodulin-dependent protein kinase II (pCaMKII) and phosphorylated extracellular signal-regulated kinases 1/2 (pERK1/2) in both brain regions were elevated. RO5166017 prevented cocaine priming-induced increase in pCaMKII in both the PFC and the NAc, but it did not affect the level of pERK1/2. Furthermore, bilateral microinjection of RO5166017 (5 ug/ 0.5ul/ side) into the prelimbic cortex of PFC and NAc shell both inhibited cue- and drug-induced reinstatement of cocaine seeking. These results suggested that CaMKII-dependent signaling pathway within the PFC and the NAc may mediate the role of TAAR1 in reinstatement of cocaine seeking.

Disclosures: **J. Liu:** None. **J. Li:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH GRANT DA12513

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Title: nNOS-expressing interneurons: A master switch for nucleus accumbens plasticity underlying cocaine relapse

Authors: *A. W. SMITH[1], J. L. HEINSBROEK[1], M. D. SCOFIELD[1], M. R. LORANG[2], P. W. KALIVAS[1];
[1]Neurosciences, Med. Univ. of South Carolina, Charleston, SC; [2]Biol., Col. of Charleston, Charleston, SC

Abstract: Chronic cocaine exposure produces neuroplasticity within the nucleus accumbens core (NAcore) that leads to increased vulnerability to relapse, even after protracted abstinence. Relapse is associated with a transient synaptic potentiation of corticostriatal synapses in the nucleus accumbens core, and the magnitude of this potentiation is correlated with the intensity of relapse behavior. Matrix metalloproteinases (MMPs) are Zn^{2+}-dependent endopeptidases that degrade the extracellular matrix to promote synaptic plasticity, and recent work from our lab has shown that upregulated MMP activity is required for synaptic plasticity accompanying cocaine addiction. One mechanism of MMP activation is through S-nitrosylation via nitric oxide (NO). NO is synthesized in a small subset of GABAergic interneurons within the accumbens (~1%), and these neurons are characterized by the expression of neuronal nitric oxide synthase (nNOS). We hypothesized that nNOS activity and MMP S-nitrosylation is both necessary and sufficient to drive cue-induced reinstatement of cocaine seeking. In order to test this, we used NOS1-Cre transgenic mice for selective targeting of nNOS-expressing interneurons. By stimulating Ca^{2+} signaling via Cre-dependent Gq-DREADD expression selectively in nNOS-expressing interneurons, we were able to stimulate MMP activity, potentiate cue-induced reinstatement, and drive reinstatement in the absence of cues. Furthermore, Gq stimulation in nNOS-expressing interneurons induced MMP activity throughout the accumbens, and this activity was completely abolished by an nNOS inhibitor. Future experiments will determine the effects of this activity on synaptic strength, measured by AMPA:NMDA ratio. Together, these data indicate that nNOS-

expressing interneurons are a novel portal for cortical regulation of cocaine-seeking, and furthermore that they may constitute a 'master switch' for plasticity on medium spiny neurons that underlies relapse to drug seeking.

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Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R015369

NIH Grant DA003906

NIDA Grant T32 DA 7288-22)

Title: Electrochemical detection of glutamate- and Gq-dreadd-evoked nitric oxide release in the nucleus accumbens

Authors: *M. D. SCOFIELD[1,2], A. W. SMITH[2], C. D. GIPSON[2], H. A. BOGER[2], P. W. KALIVAS[2];
[1]Neurosci., [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: The gaseous transmitter nitric oxide (NO) is produced in the nucleus accumbens core (NAcore) by a subpopulation of interneurons that express neuronal nitric oxide synthase (nNOS). Among other things, NO plays a critical role in the nitrosylation and activation of matrix metalloproteinases (MMPs) required for dendritic spine head (dh) expansion on medium spiny neurons (MSNs), linked to cue-induced cocaine seeking. We have shown that cocaine exposure enhances activity of the nNOS enzyme in the NAcore leading to the nitrosylation of MMPs, while inhibition of nNOS inhibits cue-induced activation of MMPs and cocaine seeking. Further, we show here that infusion of the mGluR5 agonist CHPG into the NAcore produced reinstated drug seeking, which was blocked by the co-infusion of nNOS inhibitor N-Propyl-L-Arginine (NPLA). In order to validate that activation of NAcore nitrergic interneurons translates to NO release in real time, evoked NO levels were measured in anesthetized animals using Nafion + o-PD coated S2 multi electrode arrays and the Quanteon FAST16mkII system. Puff application of glutamate or CHPG produced a reproducible dose-dependent increase in NO release in the

NAcore, which was inhibited by the mGluR5 antagonist MTEP or NPLA, respectively. Moreover, NO efflux was dose-dependently evoked by stimulation of Gq-coupled designer receptors exclusively activated by designer drugs (DREADDs), selectively expressed in NAcore nitrergic interneurons. Taken together, our results demonstrate that activation of glutamate receptors (including mGluR5) in the NAcore produced NO release. Further, we show that activation of Gq-signaling specifically in NAcore nitrergic interneurons also induced NO release. Combined, these data indicate that activation of nitrergic interneurons, and the subsequent NO release, is a crucial step in the signal transduction cascade between cue-induced glutamate release in the NAcore and the activation of MMPs and increased dh associated with cued cocaine seeking.

Disclosures: M.D. Scofield: None. **A.W. Smith:** None. **C.D. Gipson:** None. **H.A. Boger:** None. **P.W. Kalivas:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Title: Integrins and integrin linked kinase as a signaling pathway for mmp-9 induction of transient synaptic plasticity in cocaine relapse

Authors: *C. GARCIA-KELLER, S. SPENCER, M. D. SCOEFIELD, A. N. PHOENIX, P. W. KALIVAS;
Neurosci., Med. Univ. of South Caroline, Charleston, SC

Abstract: Chronic cocaine exposure produces neuroplasticity within the nucleus accumbens core (NAcore) that leads to increased vulnerability to relapse, even after protracted abstinence. Matrix metalloproteinases (MMPs) are inducible endopeptidases that degrade extracellular matrix (ECM) proteins (such as fibronectin, laminin and thrombospondin), as well as non-ECM signaling molecules, and reveal an RGD domain that binds and signals through integrins. Integrins are heterodimeric transmembrane cell adhesion receptors composed of subunits αβ, and their primary signaling kinase is the integrin linked kinase (ILK). A variety of data support integrin in the accumbens as a possible signaling mechanism for addictive behaviors. Previous results from our lab show that β3 integrin is upregulated by cocaine self-administration and its stimulation promotes changes in spine morphology and AMPA receptor trafficking. Moreover, administering an RGD-containing peptide into the NAcore inhibits cocaine-induced

reinstatement. It has also been shown by others that MMP-9 activation produces changes in spine morphology and NMDA receptor surface diffusion by signaling through β1 integrins in the hippocampus. We know that chronic cocaine exposure increases activity of MMP-9 and this promotes transient synaptic plasticity (t-SP: increases in spine head diameter and AMPA/NMDA) and reinstatement of drug seeking. We hypothesized that β1 and β3 integrin signaling through ILK are important in order to promote synaptic growth and regulate actin polymerization and AMPA receptor trafficking during t-SP. In order to support this hypothesis, we reduced integrin and ILK protein using an antisense morpholino and evaluated the capacity of both to mediate cue-reinstated behavior. Our preliminary studies with knock-down of NAcore levels of B1 integrin and ILK is contrary to our hypothesis, and a reduction in cue-induced reinstatement of cocaine seeking was observed in morpholino treated versus control rats. However, this may occur because the knockdown of B1 integrin caused a compensatory upregulation of ILK. We are conducting further studies us small molecule antagonists of ILK to evaluate the role of integrin signaling on reinstated cocaine seeking.

Disclosures: **C. Garcia-Keller:** A. Employment/Salary (full or part-time):; Medical University of South caroline. 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 DA003906, NIH grant DA012513. **S. Spencer:** A. Employment/Salary (full or part-time):; Medical University of South caroline. 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 DA003906, NIH grant DA012513. **M.D. Scoefield:** A. Employment/Salary (full or part-time):; Medical University of South caroline. 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 DA003906, NIH grant DA012513. **A.N. Phoenix:** None. **P.W. Kalivas:** A. Employment/Salary (full or part-time):; Medical University of South caroline. 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 DA003906, NIH grant DA012513.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA034684

NIH grant DA037216

Title: Overexpression of acid-sensing ion channel 1A in the nucleus accumbens core potentiates cocaine-seeking, but not food-seeking, behavior in rats

Authors: *V. A. MULLER EWALD[1], C. V. COSME[2], A. L. GUTMAN[2], W. R. WORTH[2], M. NOTERMAN[3], Y. LU[4], J. A. WEMMIE[4], R. T. LALUMIERE[2];
[2]Psychology, [3]Interdisciplinary Neurosci. Program, [4]Psychiatry, [1]Univ. of Iowa, Iowa City, IA

Abstract: Recent evidence indicates that acid-sensing ion channels (ASICs) in the nucleus accumbens influence conditioned place preference for cocaine and cocaine self-administration. However, whether ASICs are involved in regulating cocaine-seeking behavior is unknown. To address this issue, male Sprague-Dawley rats underwent surgery for intravenous catheter implantation and implantation of bilateral cannulas aimed at the nucleus accumbens core. The rats then underwent cocaine self-administration for a minimum of 12 d, in which active lever presses produced an intravenous infusion of cocaine and a light-tone combination of cues. Upon completion of self-administration, rats received microinjections of adeno-associated virus (AAV) into the nucleus accumbens core to produce overexpression of the ASIC1A subunit or GFP alone (control group). Groups were matched based on self-administration behavior (cocaine infusions and active lever pressing). The rats then remained in their homecages for 3 weeks in order to ensure robust ASIC1A overexpression as well as mimic procedures necessary for the incubation of craving. Rats were then returned to the operant chambers to begin drug-seeking testing. In the first session, rats underwent a cue-induced drug-seeking session, in which active lever presses produced the cocaine-associated cues. ASIC1A overexpression induced significantly more active lever pressing than their control GFP-alone counterparts. Rats then underwent a minimum of 7 d of extinction, during which active lever presses had no consequences, in order to extinguish their lever pressing. Rats overexpressing ASIC1A had more active lever presses on day 1 of extinction and required more extinction sessions in order to reach criterion. Subsequent reinstatement testing using either cues, a cocaine-prime, or a combination of cues with a cocaine-prime revealed similar effects, as rats overexpressing ASIC1A had higher levels of cocaine-seeking behavior. A subsequent experiment replicated the behavioral procedures but with food self-administration and food-seeking behavior. In this case, ASIC1A overexpression in the nucleus accumbens core had no effect on any measure of food-seeking behavior. Together, these findings indicate that overexpression of ASIC1A in the nucleus accumbens core potentiates cocaine-seeking behavior and that this effect does not appear to generalize to non-drug reward-seeking behavior. The present results suggest that targeting ASICs, and particularly the ASIC1A subunit, may be an effective and selective method for altering cocaine seeking.

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Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA015214

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Title: Systemic administration of a kainate receptor antagonist attenuates cocaine seeking and alcohol preference in rats

Authors: ***D. VAN NEST**, N. HERNANDEZ, J. MAURER, M. DE BIASI, H. R. KRANZLER, H. D. SCHIMDT, R. C. PIERCE;
Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Cocaine addiction in the United States continues to be a large public health concern for which there are no FDA-approved pharmacological treatments. Ionotropic glutamate receptors play an important role in cocaine-seeking behavior. To date, the vast majority of studies of the role of ionotropic glutamate receptors in cocaine addiction have been limited to AMPA receptor function primarily because of a lack of available drugs that selectively target kainate receptors. Thus, the role of kainate receptors in cocaine-seeking behavior remains unclear. Here, we examined the role of kainate receptors in cocaine seeking using novel pharmacological antagonists of kainate receptors. Rats were allowed to self-administer cocaine (0.25 mg/infusion i.v.) for 21 days. Cocaine self-administration was extinguished by replacing cocaine with saline. Rats were then given an acute systemic injection of cocaine (10 mg/kg, i.p.) to assess their drug-seeking behavior. In subsequent reinstatement tests, rats were pretreated with the selective kainate receptor antagonist UBP302 (0.5, 1, 5, 10 mg/kg, i.p.) or LY466195 (4, 10 mg/kg, i.p.) followed by a priming injection of cocaine. Systemic administration of LY466195 attenuated the ability of an acute priming injection of cocaine to reinstate drug-seeking behavior. In a separate set of experiments, the effect of LY466195 on alcohol consumption was assessed using the intermittent two-bottle choice paradigm. Preliminary results indicate that LY466195 (4, 10 mg/kg, i.p.) administration showed a strong trend towards reducing alcohol preference.

Together, these results suggest that kainate receptor antagonists may be useful in the treatment of cocaine craving and may influence alcohol intake as well.

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Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Program#/Poster#: 51.10/G2

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA T32-DA07290 to A.L.L.

NIDA DA08227 to D.W.S.

Title: The role of projections from the nucleus accumbens shell to the ventral pallidum in mood and motivation for cocaine

Authors: ***A. L. LORIAUX**, D. W. SELF;
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Abstract: Cocaine users often cite negative affect as a key factor behind relapse. However, the relevant neurocircuitry behind mood and motivational changes in cocaine addiction, and their relationship to one another, is not fully understood. We have recently shown that selective stimulation of nucleus accumbens shell (NAcSh) neural projections to the lateral hypothalamus (LH) increases motivation for cocaine, while producing depression-like despair in rats trained to self-administer cocaine. Global stimulation of NAcSh cell bodies also produces despair, but decreases motivation for cocaine, suggesting that other NAcSh outputs may override the motivational effects of NAcSh-LH terminal stimulation. Activity in NAc projections to ventral pallidum (VP) has been associated with depressed mood in drug naïve animals, but with conflicting effects on the motivation for cocaine. In the present study, we used a target-specific optogenetic approach to selectively activate NAcSh projections to the VP in male rats. We tested the hypothesis that increased activity in the NAcSh-VP depresses both mood and motivation for cocaine, and, thus, is behaviorally differentiated from the effects of NAcSh-LH projections. Rats were bilaterally injected with either AAV2-hSyn-hChR2(H134)-EYFP or AAV2-hSyn-EYFP

control virus into the NAcSh, and implanted with optic fibers in the terminal fields of the NAcSh in the VP. Rats were trained to self-administer intravenous cocaine (0.5 mg/kg/infusion, i.v.) 4 h/day for 3 weeks. We measured the effect of laser stimulation of the NAcSh-VP pathway (30 min pretreatment, 10 sec/min, 20 Hz, 50 mW) on motivation for cocaine as assessed by 1) performance on a progressive ratio (PR) schedule of reinforcement for cocaine, 2) drug-paired lever presses under extinction conditions and 3) cocaine-primed reinstatement. We measured behavioral despair and anhedonia with the forced swim and sucrose preference tests, respectively. Optogenetic stimulation of NAcSh-VP terminals significantly decreased the effort to self-administer cocaine in ChR2 animals compared to eYFP controls, as indicated by 43.6% lower breakpoints. ChR2 animals also had a 43.6% reduction in lever presses during early extinction and a 32.9% decrease during reinstatement compared to eYFP controls. However, in contrast to global stimulation of the NAcSh, we found no differences in measures of immobility in the forced swim test, or a difference in preference for a 1% sucrose solution. These findings suggest that activity in the NAcSh-VP circuit may decrease motivation for cocaine independent of changes in mood, and thus may serve as a possible neural substrate for addiction treatment.

Disclosures: A.L. Loriaux: None. **D.W. Self:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 51.11/G3

Topic: C.17. Drugs of Abuse and Addiction

Support: 5 P01 DA 08227

T-32 DA007290

Title: Loss of BDNF-TrkB-PLCγ signaling in accumbens shell neurons attenuates cocaine-induced dendritic spine formation

Authors: *E. M. ANDERSON[1], A. WISSMAN[1], D. GUZMAN[1], C. COWAN[2], D. SELF[1]; [1]UT Southwestern, Dallas, TX; [2]McLean Hosp., Belmont, MA

Abstract: Chronic cocaine induces dendritic spine growth in accumbens shell (NACsh) neurons, but this effect has not been shown to directly enhance addictive behavior. Spine formation has been functionally linked to BDNF-TrkB signaling in other brain regions, but whether this mechanism underlies cocaine-induced spine formation is unknown. We previously found that

cocaine induces BDNF activation of TrkB signaling through PLC. In this study, we tested the necessity of BDNF-TrkB-PLC signaling on dendritic spine formation in NACsh neurons, and compared effects with modulation of cocaine self-administration behavior. We constructed a novel HSV bicistronic vector expressing both GFP and a mutated TrkB that selectively blocks endogenous TrkB-PLC signaling (HSV-TrkB^{Y816F}), while preserving other TrkB signaling pathways. Rats implanted with bilateral NACsh cannulae were trained to self-administer (SA) cocaine on a fixed ratio (FR) schedule for 3-4 weeks, and dose-response for cocaine SA was assessed before, during, and after transient HSV expression TrkB^{Y816F} or GFP-only controls. A second HSV infusion was followed by assessment of motivation for cocaine on a progressive ratio reinforcement schedule (PR). For morphological analysis, separate cohorts engaged in cocaine or saline SA for 3 weeks, and HSVs were infused into the NACsh followed by 2 more days of SA and 24 h withdrawal prior to brain perfusion. Dendritic spine densities were quantified from confocal images of GFP-labeled neurons using Volocity 3D analysis. TrkB^{Y816F} expression caused a transient leftward shift in the dose threshold necessary to maintain cocaine SA compared with GFP controls, indicating increased sensitivity to cocaine reinforcement with loss of endogenous TrkB-PLC signaling. TrkB^{Y816F} expression also increased motivation for cocaine as assessed by breakpoints on the PR reinforcement schedule. Chronic cocaine SA increased dendritic spine densities in NACsh neurons compared to tissue from saline SA animals, similar to previous reports. In contrast, TrkB^{Y816F} expression during SA reversed cocaine-induced increases in spine density without affecting basal spine density in saline SA animals. These results are the first to implicate BDNF-TrkB activity in cocaine-induced morphological changes in the NACsh, and suggest that the TrkB-PLC signaling pathway is important for this effect. Since inhibiting this TrkB-PLC pathway also enhances the motivation for cocaine, cocaine-induced dendritic spine formation may not functionally contribute to cocaine addiction, and could represent a counter-adaptation process that reduces addictive behavior.

Disclosures: **E.M. Anderson:** None. **A. Wissman:** None. **D. Guzman:** None. **C. Cowan:** None. **D. Self:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 51.12/G4

Topic: C.17. Drugs of Abuse and Addiction

Title: Vagus nerve stimulation modulates plasticity in the prefrontal cortex-amygdala pathway and enhances extinction of drug-seeking behavior

Authors: *J. PRUETT, E. NICKEL, J. DELEON, S. KROENER;
Univ. of Texas At Dallas, Sch. of Behavioral and Brain Sci., Richardson, TX

Abstract: Cocaine addiction can cause maladaptive neuroplasticity that persists long after cessation of drug taking. The relative permanence of cue associations formed during drug taking contributes to the difficulties in treating addiction. Exposure to these cues can trigger relapse to drug use. Breaking the cue/drug association via extinction learning is one approach to prevent relapse. Vagus nerve stimulation (VNS) has previously been shown to enhance extinction of conditioned fear and to alter neural plasticity in fronto-limbic circuits. Here we trained animals to self-administer cocaine and extinguished them in the presence or absence of VNS. Two stimulation parameters were tested: Non-contingent VNS (30 seconds every 5 minutes, 500 μs pulse width, 30 Hz, 0.4 mA) or contingent VNS (500 ms every active lever press, 500 μs pulse width, 30 Hz, 0.8 mA). VNS-treatment under both conditions increased rates of extinction and reduced cue-induced reinstatement. In control experiments we delivered VNS during extinction from food self-administration and found that VNS similarly facilitated extinction from food-seeking. Lastly, VNS administered concurrently with either food or drug self-administration did not impact rates of response, suggesting that VNS does not affect appetitive behaviors. After reinstatement, we recorded local field potential recordings in the basolateral amygdala (BLA) of anesthetized animals to study VNS-induced changes in plasticity in the pathway between the medial prefrontal cortex (PFC) and the BLA. Stimulation of the infralimbic PFC (900 pulses at 1Hz) induced LTD in naïve and sham-stimulated animals, but caused no change in VNS animals, suggesting that VNS can reduce reinstatement by modulating the projection from the infralimbic PFC to the BLA. We further studied VNS modulation of the circuitry associated with drug-seeking and extinction learning by quantifying expression of activated cAMP response element-binding protein (pCREB) immediately after reinstatement in the mPFC, BLA, and nucleus accumbens. We found differential expression patterns in naïve, Sham-VNS, VNS, and Drug Only (animals that went through self-administration but not extinction) conditions. Because both contingent and non-contingent VNS facilitated extinction learning we are currently testing the effects of VNS on extinction of conditioned place preference to differentiate between effects on operant responding and contextual cues.

Disclosures: **J. Pruett:** None. **E. Nickel:** None. **J. DeLeon:** None. **S. Kroener:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 51.13/G5

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant R00DA031790

Title: Riluzole impairs reinstatement to cocaine seeking

Authors: *M. T. SEPULVEDA-ORENGO, A. C. AURIEMMA, K. L. HEALEY, J. A. ROJAS, K. J. REISSNER;
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Abstract: Cocaine abuse alters cellular dynamics within several regions of the brain's reward circuitry, including the ventral tegmental area, nucleus accumbens and prefrontal cortex (PFC), among others. Elucidation of these cellular adaptations can help identify pharmacotherapeutic candidates for cocaine addiction. One example of such a candidate is the glutamate transporter GLT-1/EAAT2, which is decreased in the nucleus accumbens following cocaine experience. GLT-1 is responsible for approximately 90% of glutamate uptake in the brain, and is critical for neuroprotection and fidelity of synaptic processing. Previous studies have reported that compounds which restore expression of GLT-1 can also reduce behavioral measures of drug seeking. Thus, we wished to test the hypothesis that another known regulator of GLT-1, riluzole, might also reduce cocaine seeking. Riluzole is an FDA approved drug for Amyotrophic Lateral Sclerosis (ALS), which decreases neuronal activity by blocking voltage-gated sodium channels. In addition, riluzole upregulates expression of GLT-1 *in vitro* and *in vivo*, leading to increased glutamate uptake by astrocytes. To determine whether riluzole has an effect on cocaine seeking, we employed the rat self-administration/extinction/reinstatement model of cocaine abuse. During the extinction phase, rats received chronic intraperitoneal injections of vehicle or riluzole (1 or 4 mg/kg), thirty min before each session. We observed a dose-dependent reduction in cue- and cocaine-primed reinstatement to cocaine. However, riluzole had no effect on cue-primed reinstatement of sucrose seeking. In addition, we recorded intrinsic excitability in prefrontal cortical neurons using whole-cell patch clamp electrophysiology in slices of rats trained to self-administer cocaine or saline, receiving chronic riluzole or vehicle injections during extinction. Preliminary data indicate a cocaine-dependent increase in prelimbic neuron excitability, which is reversed by administration of riluzole. Surprisingly, preliminary results also indicate that riluzole administration results in an increase in excitability in infralimbic neurons. These results suggest that riluzole restores levels of intrinsic excitability in the prefrontal cortex, which may contribute to its effect on cocaine seeking. These results further support an existing body of literature which implicates GLT-1 regulators as therapeutic candidates for psychostimulant addiction.

Disclosures: M.T. Sepulveda-Orengo: None. **A.C. Auriemma:** None. **K.L. Healey:** None. **J.A. Rojas:** None. **K.J. Reissner:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

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Program#/Poster#: 51.14/G6

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA DA031790

NIDA T32-DA07244

Title: Regulation of glutamate transporter-1 gene expression by cocaine self-administration and withdrawal

Authors: *R. KIM, M. T. SEPULVEDA-ORENGO, K. J. REISSNER;
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Abstract: Relapse to cocaine abuse is characterized by alterations in glutamatergic signaling in the nucleus accumbens (NAc). For example, glutamate levels are increased in the NAc during reinstatement to cocaine seeking in the rat. Glutamate uptake is maintained primarily by the astroglial glutamate transporter GLT-1, which accounts for up to 90% of glutamate clearance from the synapse. GLT-1 protein expression and function is decreased in the NAc following cocaine self-administration and withdrawal, and this decrease is correlated with both cocaine exposure and length of withdrawal. Moreover, restored expression of GLT-1 is a necessary component of compounds which block reinstatement to cocaine, including N-acetylcysteine and propentofylline. However, the mechanism responsible for regulation of GLT-1 protein by cocaine remains unclear. To examine this question, rats were trained to self-administer IV cocaine or saline in a short access (SA, 2 hours) or long access (LA, 6 hours) paradigm. Following 2 weeks of SA to cocaine (n=8) or saline (n=8), rats underwent extinction training in the operant chambers for 3 weeks. In contrast, following 10 days of LA to cocaine (n=8) or saline (n=7), rats remained abstinent in the home cage for 45 days. Twenty four hours following the last extinction session (SA rats) or last day of abstinence (LA rats), tissue was harvested from the NAc and prelimbic cortex (PL), a region known to have important projections to the NAc. Gene expression was then examined in these two regions using qRT-PCR and primers specific for two GLT-1 splice variants (GLT-1A and GLT-1B). In SA rats, no differences in GLT-1A or GLT-1B mRNA levels were found between cocaine and saline rats in NAc or PL samples. In the NAc of LA rats, a significant decrease was found in GLT-1A gene expression in cocaine vs. saline rats ($t(13) = 2.735$, $p < .05$), but no difference was found in GLT-1B gene expression. In the PL of LA rats, we observed a trend toward a decrease in GLT-1A and a statistically

significant decrease in GLT-1B gene expression ($t(13) = 2.527$, $p < .05$). These results show that the decrease in GLT-1 protein after SA to cocaine and extinction may not be due to a decrease in gene expression, and may reflect protein degradation and/or trafficking. Furthermore, prolonged abstinence after LA cocaine self-administration induces specific changes in GLT-1 gene expression that differs amongst the GLT-1 splice variants, and differs between PL and NAc. Future studies will be designed to investigate the mechanism of the identified genetic suppression of GLT-1 following LA to cocaine.

Disclosures: R. Kim: None. **M.T. Sepulveda-Orengo:** None. **K.J. Reissner:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 51.15/G7

Topic: C.17. Drugs of Abuse and Addiction

Title: Subpopulations of adenosine a2a receptors in the nucleus accumbens play different roles in cocaine seeking

Authors: *N. HAYNES, C. E. O'NEILL, S. LEVIS, D. SCHREINER, J. STAFFORD, R. K. BACHTELL;
Psychology and Neurosci., Univ. of Colorado, Boulder, CO

Abstract: Repeated cocaine administration produces perturbations in dopamine and glutamate neurotransmission that contribute to the reinstatement of extinguished cocaine seeking. The synaptic localization of adenosine A2A Receptors (A2AR) can influence both dopamine and glutamate neurotransmission to have a differential roles on behavior. Thus, A2AR's are localized on presynaptic glutamate terminals that oppose direct pathway medium spiny neurons, while postsynaptic A2AR's are localized on indirect pathway neurons. These studies sought to elucidate differential effects of these subpopulations of A2AR's on cocaine seeking. Male Sprague-Dawley rats self-administered cocaine in 10 daily self-administration sessions on a fixed-ratio 1 schedule. Lever pressing was then extinguished in six daily extinction sessions. We first tested whether presynaptic and postsynaptic A2AR antagonism (SCH 442416 and KW 6002, respectively) was sufficient to reinstate cocaine seeking. We next tested the effects of SCH 442416 and KW 6002 on reinstatement to cocaine seeking induced by systemic cocaine or prefrontal cortex (PFC) stimulation. We found that KW 6002 induced reinstatement of cocaine seeking and facilitated cocaine-induced seeking. Conversely, SCH 442416 had no effect on cocaine seeking alone and blunted cocaine-induced seeking. We saw similar bidirectional effects

when cocaine seeking was induced by pharmacological stimulation (cocaine or AMPA) of the PFC. Analysis of the phosphorylation state of the presynaptic marker, synapsin, suggests that presynaptic A2AR antagonism inhibits PFC-NAC neurotransmission. Together, the data suggest that postsynaptic A2AR antagonisms facilitates reinstatement via disinhibiting dopamine D2 receptor activity on indirect pathway neurons while presynaptic A2AR antagonism inhibits reinstatement by reducing glutamate neurotransmission onto direct pathway neurons. This research helps clarify differential roles of A2AR subpopulations in the NAC in cocaine addiction.

Disclosures: **N. Haynes:** None. **C.E. O'Neill:** None. **S. Levis:** None. **D. Schreiner:** None. **J. Stafford:** None. **R.K. Bachtell:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA33641

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Title: Deep brain stimulation (DBS) of nucleus accumbens afferent structures attenuates the reinstatement of cocaine seeking

Authors: *L. A. GUERCIO, H. SCHMIDT, R. PIERCE;
Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Cocaine abuse is a major public health concern, with more than 2 million current users in the United States alone. One of the major obstacles in treating cocaine addiction is the discouragingly high rate of relapse after detoxification. Drug craving and relapse to cocaine seeking are modeled in rodents using the reinstatement paradigm. Deep brain stimulation (DBS) is an FDA-approved treatment for movement disorders. The success of DBS in treating movement disorders has paved the way for its examination as a therapeutic modality in many psychiatric disorders, including drug addiction. Previous work has shown that DBS of the

nucleus accumbens shell attenuates priming- and cue-induced reinstatement of cocaine seeking, likely due to modulation of efferent glutamatergic projections from the mesocorticolimbic system. The medial prefrontal cortex (mPFC), ventral hippocampus (vHipp), and basolateral amygdala (BLA) send strong glutamatergic projections to the nucleus accumbens and have been shown to be critically involved in cocaine seeking. Here, we investigated the effects of DBS in these nuclei on the reinstatement of cocaine seeking. Initially, rats were allowed to press a lever for cocaine (0.254 mg/59 μL, i.v.) using a fixed-ratio 5 (FR5) schedule of reinforcement. After 21 days of cocaine self-administration, responding was extinguished by substituting saline for cocaine. Following extinction, we assessed cocaine priming-induced reinstatement of drug seeking. During reinstatement test sessions, DBS was administered bilaterally to each nucleus through bipolar stainless steel electrodes. Our findings show that DBS of the mPFC, vHipp, and BLA attenuates the reinstatement of cocaine seeking in rats. These results suggest a role for DBS as a possible therapeutic modality for cocaine addiction and relapse.

Disclosures: **L.A. Guercio:** None. **H. Schmidt:** None. **R. Pierce:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: 1R01DA029122-03 (AMR)

5T32DA7274-20 (KCS)

5 F31 DA032169-03 (ASL)

Title: Cav1.2 channel-mediated regulation of GluA1 phosphorylation and trafficking in the hippocampus is essential for extinction of cocaine conditioned place preference

Authors: *C. BURGDORF[1], K. C. SCHIERBERL[1], A. S. LEE[1], F. HOFMANN[2], R. L. HUGANIR[3], A. M. RAJADHYAKSHA[1];
[1]Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY; [2]Tech. Univ. Munich, Munich, Germany; [3]Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Cocaine addiction is a chronic, life-long relapsing disorder. To date, no treatments have been effective in extinction of cocaine seeking behavior. Cav1.2 L-type Ca2+ channels

(LTCCs) are necessary for cocaine-induced behaviors, LTP and long-term memory. Cocaine exposure also modifies glutamatergic synapses in regions innervated by dopaminergic inputs through the regulated trafficking of AMPA receptor (AMPAR) localization. Trafficking of the GluA1 AMPAR subunit to the neuronal postsynaptic density (PSD) is regulated by changes in phosphorylation at its serine 831 (S831) residue, a CaM kinase II (CaMKII)-dependent site and its serine 845 (S845) residue, a protein kinase A (PKA) site. Previous findings from our lab has shown that Cav1.2 LTCCs increase cell surface GluA1 levels via CaMKII phosphorylation of S831 in the nucleus accumbens of cocaine sensitized mice. However, the role of Cav1.2 and Cav1.2-mediated GluA1 trafficking in extinction of cocaine seeking behavior remains unknown. Thus, in this study, we employed the cocaine conditioned preference (CPP) model in combination with genetic and molecular techniques to address this question. Using mice lacking Cav1.2 in dopamine D1-containing neurons we found Cav1.2 in the hippocampus to be required for extinction of cocaine CPP. Site-specific knockout of Cav1.2 using AAV-Cre identified a role of Cav1.2 specifically in the hippocampus in cocaine extinction. In cocaine extinguished C57BL/6 mice, there was an increase in S831 P-GluA1 and total GluA1 levels at the PSD in the hippocampus with no change in S845. To directly test the requirement of GluA1 phosphorylation in cocaine extinction, phosphomutant mice with a serine to alanine substitution (S831A or S845A) were utilized. While both genotypes acquired cocaine CPP, both S831A and S845A phosphomutant mice were unable to extinguish cocaine place preference, demonstrating a critical role for S831 and S845 in cocaine extinction. Subcellular fractionation of hippocampus tissue and western blot analyses found no difference in total GluA1 levels in the cytoplasmic fractions of S831A and S845A mutant mice compared to their respective wild-type littermates. However, lower levels of GluA1 were found in the synaptosomal fractions of both phosphomutant mice compared to wild-type littermates. Experiments examining GluA1 levels in PSD fractions and intracellular mechanisms of Cav1.2 regulation of GluA1 trafficking are currently ongoing. In summary, we have identified a role of Cav1.2 channels in dopamine D1 receptor-containing neurons of the hippocampus in extinction of cocaine CPP via regulation of GluA1 phosphorylation-dependent trafficking.

Disclosures: C. Burgdorf: None. **K.C. Schierberl:** None. **A.S. Lee:** None. **F. Hofmann:** None. **R.L. Huganir:** None. **A.M. Rajadhyaksha:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: 1R01DA029122-03

Title: Cav1.3 L-type Ca^{2+} channels: Role in VTA dopamine neurons on cocaine's behavioral effects and genetic variants in cocaine dependent humans

Authors: *A. MARTINEZ[1,2], J. HAO[2], R. RICE[2], J. STRIESSNIG[3,4], S. HAN[5], A. M. RAJADHYAKSHA[1,2];
[1]Pediatric Neurol., Weill Cornell Med. Col., New York, NY; [2]Dept. of Pediatrics, Div. of Pediatric Neurol., New York Presbyterian Hosp., New York, NY; [3]Pharmacol. and Toxicology, [4]Ctr. for Mol. Biosci., Univ. of Innsbruck, Innsbruck, Austria; [5]Psychiatry, Univ. of Iowa, Iowa, IA

Abstract: It is well established that Ca2+ neurotransmission through glutamate receptors within the reward pathway regulates cocaine addiction-related behaviors. Most recently, another route of calcium neurotransmission through L-type calcium channels (LTCCs), has been gaining prominence in the field of addiction. Two subtypes of LTCCs are expressed in the brain, Cav1.2 and Cav1.3, important mediators of CREB activation and CREB-mediated gene expression. We previously reported that Cav1.2 channels mediate the expression of cocaine psychomotor sensitization while Cav1.3 channels, the development (Giordano et al. 2010). In addition, we showed that nifedipine (LTCC blocker) attenuates acute cocaine-induced phosphorylation of CREB (P-CREB) in the nucleus accumbens. However, the specific contribution of the two LTCC subtypes in other cocaine behavioral protocols with greater clinical relevance, like cocaine conditioned place preference (CPP) and cocaine self-administration still remains unknown. Since it has been found that Cav1.3 is more abundant in the VTA (Rajadhyaksha et al. 2004), we investigated the role of VTA Cav1.3 in cocaine CPP. In the present study, using mutant mice expressing 1,4-dihydropyridines (DHP)- insensitive Cav1.2 (Cav1.2DHP-/-) treated with nifedipine or Cav1.3shRNA in the VTA, we found that Cav1.3 is necessary for the acquisition of cocaine CPP and not consolidation or expression. Molecular studies have revealed that Cav1.3 mediates acute cocaine-induced P-CREB in the VTA via activation of CaMKinase II. Treatment with KN93 (CaM kinase II inhibitor) in the VTA of wild type C57BL/6J mice blocked the acquisition of cocaine CPP. Additional studies are currently underway to identify the pathway that signals from Cav1.3/CamKII to CREB in response to cocaine. Recently, it has been shown in neuronal cultures that Ca2+ influx via Cav1.3 translocates γCaMKII into the nucleus to shuttle Ca2+/CaM for the induction of P-CREB (Huan Ma et al. 2014). Our preliminary results find that acute cocaine increases nuclear levels of γCaMKII and P-CREB in the VTA. Ongoing studies are examining the role of Cav1.3 channels in cocaine-induced nuclear translocation of γCaMKII. Our preclinical Cav1.3 findings in cocaine behaviors is further supported by human GWAS study. Examination of 947 single nucleotide polymorphisms (SNPs) within the gene for Cav1.3 (CACNA1D) has identified three significant SNPs associated with cocaine dependence. Taken together, these findings suggest that VTA Cav1.3 channel-activated pathway plays an

important role for the acquisition of cocaine CPP, a measure of cocaine reward that may contribute to cocaine addiction seen in humans.

Disclosures: **A. Martinez:** None. **J. Hao:** None. **R. Rice:** None. **J. Striessnig:** None. **S. Han:** None. **A.M. Rajadhyaksha:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DA038048

Title: L-type calcium channels in the ventral tegmental area mediate cue-induced cocaine-seeking

Authors: ***E. J. NUNES**[1], S. M. HUGHLEY[1], W. SOLECKI[1], A. M. RAJADHYAKSHA[4], N. A. ADDY[1,2,3];
[1]Psychiatry, [2]Interdepartmental Neurosci. Program, [3]Dept. of Cell. and Mol. Physiol., Yale Univ., New Haven, CT; [4]Pediatric Neurol., Weill Cornell Med. Col., New York, NY

Abstract: Exposure to drug associated cues promotes drug-seeking behavior and is a challenge for the treatment of drug addiction. The presentation of cues induces burst firing in ventral tegmental area (VTA) DA neurons and subsequent phasic DA release in the nucleus accumbens (NAc) that promotes drug-seeking behavior. Nicotinic and muscarinic receptors in the VTA are critical regulators of burst firing in the VTA and phasic DA release in NAc. Recent data from our laboratory has demonstrated that pharmacological blockade of VTA cholinergic receptors decreases phasic DA release in NAc and reduces cue-induced drug seeking in cocaine withdrawn rats. Importantly, L-type calcium channels (LTCCs) are also expressed on VTA DA neurons and interact with cholinergic receptors to regulate burst firing of VTA DA neurons. However, the role of VTA LTCCs in cue-induced drug seeking are unknown. Here, we sought to determine if pharmacological blockade of LTCCs in the VTA would alter cue-induced drug-seeking following cocaine withdrawal. Male Sprague-Dawley rats underwent 10 days of IV cocaine (0.5 mg/kg/inf) self-administration training, where active lever response resulted in intravenous cocaine delivery in the presence of a compound cue (tone + light), and inactive lever response had no programmed consequence. Following a withdrawal period of 10 days, with no exposure to cocaine or the cues, rats were tested for cue-induced cocaine-seeking, where active lever

response resulted in the presentation of the compound cue alone, in absence of cocaine delivery. VTA infusion of the LTCC antagonist, nifedipine (10 μg/side) reduced cue-induced cocaine seeking on withdrawal day 10 (WD 10). In parallel experiments, where male Sprague-Dawley rats were trained to self-administer sucrose pellets, VTA infusion of nifedipine (10 μg/side) on WD10, had no effect on cue-induced sucrose seeking. In subsequent experiments, our data revealed that VTA administration of the LTCC antagonist, isradipine (288 pg/side), also reduced cue-induced cocaine-seeking on WD10. Our results thus far suggest that blockade of L-type calcium channels in VTA specifically reduces cue-induced seeking for the drug reinforcer, cocaine, but not the natural reinforcer, sucrose. Ongoing experiments are seeking to identify the role of specific LTCC subtypes and to determine whether these effects are mediated through regulation of phasic DA release.

Disclosures: E.J. Nunes: None. **S.M. Hughley:** None. **W. Solecki:** None. **A.M. Rajadhyaksha:** None. **N.A. Addy:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: Post-doctoral Study Grant Fyssen Foundation

DA 003906

DA 12513

Title: Role of intra-accumbens brain-derived neurotrophic factor on cue-induced reinstatement after cocaine self-administration

Authors: *A.-C. BOBADILLA, C. GARCIA-KELLER, S. MCWHIRTER, P. W. KALIVAS; Dept. of Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: Brain-derived Neurotrophic Factor (BDNF) has been shown to have a critical role not only on neurite growth during early stages of development, but also on physiological functions in the adult brain, as well as on maladaptive behaviors like addiction. Several studies found in the literature explored the role of BDNF in addiction-related brain regions, like the pre-frontal cortex (PFC), the ventral tegmental area (VTA) or the nucleus accumbens, both shell and core (NAcore). In adulthood, the expression of BDNF in the NAcore is low, and the two main sources

of BDNF are glutamatergic projections from the PFC and dopaminergic input from the VTA. Both D1- and D2-receptors expressing medium spiny neurons (MSNs) of the NAcore express the primary receptor for BDNF, TrkB. BDNF binding to TrkB induces activation of several intracellular signaling cascades like MAPK, PI3K, phospholipase C-γ. It has been proposed that BDNF affects on cocaine reward are mainly due to activation of TrkB on D2-expressing MSNs, since specific TrkB gene deletion induces a decrease in cocaine-induced place preference and profound neuronal firing modifications (Lobo et al., 2010). Here we seek to understand the rapid, acute effects of BDNF in the NAcore on drug seeking, using the behavioral model of cocaine self-administration in rats. To study the non-transcriptional effects of BDNF in the NAcore, we microinjected BDNF 15 min before cue-induced reinstatement. BDNF induced a clear decrease of reinstatement that seemed endure for days after administration. Conversely, we used TrkB/Fc, a soluble fusion protein that blocks BDNF binding to TrkB, to test whether blocking endogenous BDNF-induced activation of TrkB could prevent this effect. Preliminary data shows that blocking TrkB activation 15min before reinstatement potentiates reinstatement and prevents co-administration of BDNF from antagonizing reinstated cocaine seeking. These results suggest that, in addition to the long lasting transcriptional effects of BDNF shown in literature, acute activation of the TrkB intracellular pathway just before reinstatement can prevent cocaine seeking.

Disclosures: **A. Bobadilla:** A. Employment/Salary (full or part-time):; MUSC, Fyssen Foundation. **C. Garcia-Keller:** A. Employment/Salary (full or part-time):; MUSC. **S. McWhirter:** None. **P.W. Kalivas:** A. Employment/Salary (full or part-time):; MUSC. 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.; DA003906; DA12513.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 51.21/G13

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA031916

Title: Fluoxetine potentiates methylphenidate-induced behavioral stereotypies and subsequent cocaine self-administration in rats

Authors: M. MARINELLI, J. A. BEVERLEY, L. LAMOUREUX, *H. STEINER;
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Abstract: The psychostimulant methylphenidate (Ritalin) is used in the treatment of attention-deficit hyperactivity disorder (ADHD) and as a cognitive enhancer in the healthy. Methylphenidate, like cocaine, acts by blocking the reuptake of dopamine. However, unlike cocaine, methylphenidate does not affect serotonin. Serotonin contributes to addiction-related gene regulation and behavior induced by cocaine. Thus, the lack of a serotonin effect may explain methylphenidate's more moderate gene regulation and addiction liability. Indeed, our previous studies showed that enhancing serotonin, by adding a selective serotonin reuptake inhibitor (SSRI), fluoxetine, to methylphenidate, potentiates methylphenidate-induced gene regulation in the striatum and nucleus accumbens, mimicking cocaine effects. Here, we investigated behavioral correlates of these neuronal changes in adult rats. Behavior was assessed during a repeated drug pretreatment phase (6-8 days) and in response to cocaine, two weeks later. Our results show that adding fluoxetine (5 mg/kg) to methylphenidate (5 mg/kg) potentiates the increase in stereotypies over the course of the repeated pretreatment phase. This effect was particularly pronounced in a subset of rats (about 40%), which showed emerging stereotypies early-on during pretreatment. Two weeks after the pretreatment phase, rats were either given a cocaine challenge (15 mg/kg) and tested in an openfield test or started cocaine self-administration training (150 μg/kg per infusion, 2 h per day for 10 days). In the openfield test, cocaine-induced stereotypies correlated with stereotypies during pretreatment. Moreover, pretreatment with methylphenidate plus fluoxetine facilitated the acquisition of cocaine self-administration. This effect was limited to animals that showed early development of stereotypies during pretreatment. These results show enhanced behavioral responsiveness to cocaine (stereotypies, and cocaine self-administration) in a subpopulation of rats after exposure to methylphenidate plus fluoxetine. Our findings suggest that SSRIs may enhance the addiction liability of methylphenidate in a subpopulation of individuals.

Disclosures: M. Marinelli: None. **J.A. Beverley:** None. **L. Lamoureux:** None. **H. Steiner:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIDA R01DA020654-S1

Title: Food restriction stress enhances cocaine seeking and VTA dopamine neuron activity

Authors: *A. GORDON, M. MARINELLI, V. S. RAMACHANDRA;
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Abstract: Stress augments drug consumption, sensitizes animals to locomotor activation of psychomotor stimulants, and induces reinstatement of drug seeking. The interaction of stress and drug use is well documented in humans and animals; however, the effects of repeated mild stressors on drug seeking and activity of dopamine (DA) neurons within the ventral tegmental area (VTA) remain under-studied. We carried out a series of experiments to determine if the repeated mild stressor, food restriction (FR), alters basal activity of VTA DA neurons, and to determine if FR during protracted abstinence from cocaine consumption influences drug seeking behavior by a DA dependent mechanism. In the VTA DA recording experiment, rats were either fed ad libitum (control) or were food-restricted to maintain 90% of baseline body weight (FR) for an extended period of time. We then measured the basal firing rates of VTA DA neurons using *in vivo* single unit recordings under anesthesia to determine if food restriction alters VTA DA activity. In a separate group of animals, male rats were trained to self-administer cocaine (600ug/kg) i.v. for one week, and were then maintained on either control or FR diets for 9 days. Following this schedule, animals underwent a within session extinction/reinstatement (cocaine precipitated) procedure during which we measured drug seeking behavior and locomotion. Prior to this procedure, rats were injected i.p. with either a low dose of quinpirole (decrease DA neuron firing rate) or saline (control) to determine if observed effects were dependent on DA neuron activity. We discovered that FR leads to increased basal VTA DA activity. We also demonstrated that FR during protracted withdrawal increases drug seeking during extinction and reinstatement, and this increase is blocked by reducing DA neuron activity. This data adds to the literature on the interaction between stressful environmental cues and drug seeking behaviors.

Disclosures: A. Gordon: None. **M. Marinelli:** None. **V.S. Ramachandra:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Support: R21DA031577

5T32AA007471

Start up fund to M Marinelli UT Austin

Brain Research Foundation (seed grant)

Title: The LPO: role on dopaminergic transmission, drug taking, and seeking

Authors: *R. G. WILL[1], R. C. TWINING[2], V. S. RAMACHANDRA[3], M. MARINELLI[3]; [1]Psychology, Univ. of Texas Austin, Austin, TX; [2]Biomed. Sci., Marquette Univ., Milwaukee, WI; [3]Pharmacol. & Toxicology, Univ. of Texas, Austin, TX

Abstract: The lateral preoptic area of the hypothalamus (LPO) projects to the ventral tegmental area (VTA). Activity of neurons in the VTA is important for addiction-related behaviors such as cocaine taking and seeking. The goal of this study was to determine how LPO activation modulates neural response in the VTA, and dopamine responsive behaviors such as cocaine taking and seeking. Optogenetic activation of LPO projections to the VTA decreased the firing rate of GABAergic neurons and increased the firing rate of dopaminergic neurons in the VTA. Disinihibition of the LPO neurons via administration of bicuculline into the LPO facilitated cocaine seeking behavior but had no effect on cocaine taking during self-administration. Our results suggest that the LPO sends inhibitory projections to GABAergic neurons of the VTA, thereby increasing dopaminergic activity. Furthermore, disinhibition of the LPO increases cocaine seeking suggesting projections from the LPO to VTA modulates behavioral responsiveness to cocaine. This data supports the role of LPO as a novel structure involved in cocaine-seeking behavior.

Disclosures: R.G. Will: None. **R.C. Twining:** None. **V.S. Ramachandra:** None. **M. Marinelli:** None.

Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: DoD PR110146

NIH Grant DA033358

Title: Pharmacological antagonism of the toll-like receptor 4 attenuates cocaine induced reinstatement

Authors: *K. T. BROWN, C. ONEIL, S. LEVIS, T. FABISIAK, A. NORTHCUTT, L. WATKINS, R. BACHTELL;
Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Cocaine addiction is a chronic relapsing disorder characterized by persistent perturbations to an organism's homeostatic processes resulting in continued relapse vulnerability. Although considerable attention has been paid to the neuroadaptive consequences of chronic cocaine taking, few studies have examined the role of microglia, the brain's resident immune cells, in cocaine relapse. The Toll-Like Receptor 4 (TLR4) is largely expressed on microglia and is a molecular pattern receptor that recognizes xenobiotics as foreign and induces proinflammatory signaling in the central nervous system. Cocaine binds to the TLR4 complex resulting in the release of the proinflammatory cytokines interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and nuclear factor of kappa B1A (NFKB1A) in the mesocorticolimbic dopamine system. Here, we used a rodent model of cocaine addiction where male Sprague-Dawley rats self-administered cocaine in 15 daily 2-hour sessions. Following self-administration, animals underwent extinction training and were challenged with cocaine. In one experiment, tissue punches from the ventral tegmental area (VTA) and nucleus accumbens (NAc) were collected and analyzed for expression of proinflammatory cytokine mRNA. Results indicate that cocaine self-administration enhanced the expression of the proinflammatory cytokine, IL-1β. In another experiment, rats were tested in a drug-induced reinstatement paradigm where pharmacological antagonism of the TLR4 receptor with lipopolysaccharide from the bacterium Rhodobacter sphaeiroides (LPS-RS) was administered locally in the NAc and VTA. The results demonstrate that TLR4 antagonism in either the VTA or the NAc significantly reduced cocaine-primed reinstatement of drug seeking. These results are consistent with the hypothesis that cocaine-induced microglia-dependent proinflammatory signaling is involved in cocaine relapse that is characteristic of drug addiction.

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Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01DA031900

Title: The role of HCRTr1 in the VTA on Dopamine signaling; implications for addiction

Authors: *D. L. BERNSTEIN[1], C. BASS[2], K. LEVY[1], R. A. ESPAÑA[1];
[1]Neurobio. and Anat., Drexel Univ., Philadelphia, PA; [2]Immunol. and Pharmacol., Univ. of Buffalo, Bufflalo, NY

Abstract: The hypocretin / orexin (HCRT) system has been recognized to modulate motivated behavior via actions on the mesolimbic dopamine (DA) system. This generally neuroexcitatory peptide system sends extensive projections to numerous reward-related regions including the ventral tegmental area (VTA) where both the HCRT receptor 1 (HCRTr1) and HCRT receptor 2 subtypes are found. HCRT peptides have been shown to drive VTA DA cell activity, increase DA responses to cocaine in the nucleus accumbens (NAc), and promote cocaine self-administration, while blockade of HCRTr1 produces the opposite effects. Although the existing literature points to the HCRTr1 as an important regulator of reward and reinforcement processing, the majority of studies have employed acute, pharmacological manipulations of HCRT signaling. Moreover, these studies have traditionally relied on peripheral delivery of the HCRTr1 antagonist, SB-334867. Therefore, currently little is known about the long-term, modulatory role of HCRTr1 in the brain, or the specificity of actions at this receptor within the VTA. To address these issues, we knocked down HCRTr1 in the VTA and used fast scan cyclic voltammetry to measure baseline and cocaine-induced changes to DA signaling in the NAc. Further, we evaluated the effects of VTA-HCRTr1 knockdown on the acquisition and maintenance of cocaine self-administration behavior. Preliminary results suggest that long-term knockdown of VTA-HCRTr1 disrupts DA neurotransmission in the NAc under baseline and cocaine conditions, and also reduces cocaine self-administration behavior. When considered in the context of the existing literature, our experimental findings provide further support for the involvement of HCRTr1 in the VTA in regulating reward and reinforcement processes, and further suggest that HCRTr1 may be an effective target for future pharmacotherapies to treat substance abuse, particularly the abuse of cocaine.

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Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

Location: Hall A

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01DA030807 Striatal 5-HT6 receptors, reward and addiction

Title: Increased expression of 5-HT6 Receptors in the indirect pathway reduces cocaine self-administration

Authors: *M. BRODSKY[1], A. W. GIBSON[1], D. SMIRNOV[1], S. NAIR[1], J. F. NEUMAIER[2]; [2]Psychiatry and Behavioral Sci., [1]Univ. of Washington, Seattle, WA

Abstract: "Increased expression of 5-HT_6 Receptors in the indirect pathway reduces cocaine self-administration" Drug addiction affects millions of people throughout the world and contributes heavily to healthcare costs and death. The nucleus accumbens (NAc) in the mammalian ventral striatum plays a large role in addiction, particularly in motivation for drug and reward-seeking behavior. Serotonin (5-HT) neurotransmission has also been implicated in addiction, and 5-HT_6 receptors are strongly expressed in the direct and indirect pathway medium spiny neurons (MSNs), the main outputs from the NAc. While there is evidence linking these receptors to drug reward, the exact mechanism by which they influence drug-associated behavior is unknown. In the present study we used viral vectors using dynorphin- or enkephalin promoter to drive expression of 5-HT_6 receptors or enhanced green fluorescent protein (eGFP) selectively in the direct or indirect pathway MSNs of the NAc shell (NAcSh), respectively. Rats were then trained to self-administer cocaine and their responding was investigated using fixed ratio, progressive ratio, and dose-response operant reinforcement conditions. Increased 5-HT_6 receptor expression in indirect but not direct pathway MSNs changed the overall pattern of cocaine taking, reduced the amount of cocaine self-administered under fixed ratio schedules, especially at low doses, increased the time to the first response and length of the inter-infusion interval, but did not alter motivation as measured by progressive ratio "break point" analysis. We conclude that 5-HT_6 receptors in indirect pathway neurons of NAcSh increased the sensitivity to the reinforcing properties of cocaine, particularly at low doses, suggesting that these receptors may be a target for pharmacological manipulation in the treatment of cocaine addiction.

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Poster

051. Cocaine: Neural Mechanisms of Reinforcement and Relapse I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA033386

Title: Phasic dopamine release in the nucleus accumbens during cocaine self-administration under different operant requirements

Authors: *I. OLIVA, M. WANAT;
Neuroscience Institute, University of Texas at San Antonio, San Antonio, TX

Abstract: The mesocorticolimbic dopamine system plays a crucial role in the development of drug addiction. Dopamine levels are elevated in response to drug-paired cues in rats self-administering cocaine, though this has been primarily examined only under low effort conditions with a fixed ratio 1 (FR1) reinforcement schedule. A hallmark of drug addiction is the willingness to exert considerable time and effort to obtain the drugs. However, it is not known whether cue-evoked dopamine release is related to the effort exerted in rats self-administering cocaine under high effort reinforcement schedules. We utilized fast-scan cyclic voltammetry to monitor phasic dopamine release using chronically-implanted electrodes in the nucleus accumbens in rats self-administering cocaine under FR3 and progressive ratio (PR) reinforcement schedules. Upon completion of the required number of nosepokes, rats received an intravenous 300 μg/kg cocaine infusion. Cocaine administration was paired with a 5 second duration audio cue (tone) and a 20 second time out period. Our preliminary results illustrate higher dopamine release to cocaine-paired cues during the PR reinforcement schedule sessions relative to the FR3 reinforcement schedule sessions. We also employed a model to estimate the cocaine concentration in the brain during these behavioral sessions to associate the dopamine response to cocaine levels. Preliminary analysis suggests the dopamine-release towards cocaine-paired cues is not related to the cocaine concentration in the brain. These findings suggest that dopamine release toward cocaine-paired cues is sensitive to the amount of effort required to earn a drug infusion, and is not driven by the pharmacological actions of cocaine.

Disclosures: I. Oliva: None. **M. Wanat:** None.

Poster

052. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: National Basic Research Program 2015CB553501

National Natural Science Foundation 91332115, 31170988, 31400880

Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences KLMH2014ZG02

Title: Region-specific role of DNA methylation in the reconsolidation of appetitive and aversive memories associated with morphine in rats

Authors: *J.-J. ZHANG[1], P. LIU[2], N. SUI[2];
[1]Inst. of Psychology, Chinese Acad. of Scienc, Beijing, China; [2]Inst. of Psychology, Chinese Acad. of Sci., Beijing, China

Abstract: Drug-related positive and aversive memories are the main factors contributing to the persistence of relapse risk. Upon retrieval, established memories enter a transiently reconsolidation state, which is important for the persistent maintain of addiction memories. Our previous work demonstrated that DNA methylation are essential regulators in memory consolidation, but little is known about the role of DNA methylation in the memory reconsolidation. Here, we used conditioned place preference (CPP) and conditioned place aversion (CPA) paradigms to investigate the role of DNA methylation in the reconsolidation of morphine-related emotional memory. The results showed that the microinjections of DNA transmethylase inhibitors 5-aza-2-deoxycytidine into basolateral amygdala (BLA) but not central nucleus of amygdala (CeA), nucleus accumbens (NAc) shell or core immediately after memory reactivation disrupted the reconsolidation of morphine-induced CPP. Similarly, the inhibition of DNA methylation in agranular insula but not in BLA or CeA persistently disrupt morphine-naloxone induced CPA. These results indicate that the reconsolidation of drug-related positive and aversive memory is region-spcific regulated by DNA methylation.

Disclosures: **J. Zhang:** None. **P. Liu:** None. **N. Sui:** None.

Poster

052. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA038042

University of Wisconsin-Milwaukee Graduate School

Title: Blocking infralimbic basic fibroblast growth factor (bFGF or FGF2) facilitates extinction of drug seeking

Authors: *M. HAFENBREIDEL, C. RAFA TODD, C. W. SMIES, R. C. TWINING, D. MUELLER;
Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Stimulant drug use results in structural and functional changes in reward-related brain regions (Flores & Stewart, 2000). These changes may underlie the persistence of compulsive drug seeking and relapse that characterizes drug addiction. Neurotrophic factors, such as basic fibroblast growth factor (bFGF or FGF2), are necessary for neuronal survival, growth, and differentiation, and may mediate drug-induced morphological changes that underlie the perseveration of addiction. Following cocaine exposure, bFGF is increased in reward-related brain regions (Fumagalli et al., 2006), including the infralimbic medial prefrontal cortex (IL-mPFC). The IL-mPFC is necessary for extinction (Otis et al., 2014; LaLumiere et al., 2010), but whether drug-induced over-expression of bFGF in this region affects extinction is unknown. Thus, we aimed to determine if blocking bFGF in IL-mPFC would facilitate extinction following cocaine self-administration. Rats were trained to lever press for i.v. infusions of cocaine (0.25mg/inf, 90 min/day) prior to extinction. Extinction consisted of four 30 min extinction sessions, in which rats were infused into the IL-mPFC with a neutralizing antibody against bFGF that blocks the biological function of bFGF, prior to each session. Extinction retention was tested during a subsequent 90 min extinction session. Blocking bFGF in the IL-mPFC decreased lever pressing during the 90 min extinction session, indicating facilitated extinction. In contrast, blocking bFGF alone was not sufficient to facilitate extinction, as blocking bFGF and returning rats to their home cage had no effect on subsequent extinction of drug seeking. Next, we examined if bFGF or its high affinity receptor fibroblast growth factor receptor 1 (FGFR1) protein expression was altered following extinction. Rats were trained to self-administer cocaine as before with half undergoing extinction or not. Additionally, rats that were reinforced with sucrose and underwent extinction or not, rats that received yoked-saline infusions that were paired with cocaine self-administering rats (extinction or not), and a naïve home cage control were included. bFGF protein expression in the IL-mPFC was only increased following cocaine self-administration, an effect reversed by extinction. FGFR1 protein expression was not significantly altered in any group. These results suggest that cocaine-induced over-expression of bFGF in the IL-mPFC inhibits extinction, as reducing bFGF expression during extinction permits rapid extinction. Therefore, targeted reductions in bFGF during therapeutic interventions could enhance treatment outcomes for addiction.

Disclosures: M. Hafenbreidel: None. **C. Rafa Todd:** None. **C.W. Smies:** None. **R.C. Twining:** None. **D. Mueller:** None.

Poster

052. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA038042

University of Wisconsin-Milwaukee Graduate School

Title: PKA mediates prelimbic neuronal excitability underlying cocaine-associated memory retrieval

Authors: *H. YOUSUF[1], J. M. OTIS[1,2], M. K. FITZGERALD[1], D. MUELLER[1];
[1]Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Univ. of North Carolina-Chapel Hill, Chapel Hill, NC

Abstract: Persistent drug seeking is maintained by drug-associated memories, through which cues can elicit craving and relapse. Thus, impairing retrieval of drug-associated memories could dampen the motivation to seek drugs. Recently, retrieval of drug-associated memories was shown to be disrupted by inhibition of β-adrenergic receptors (β-ARs) in the prelimbic medial prefrontal cortex (PL-mPFC; Otis et al., 2013). β-ARs stimulate cAMP dependent protein kinase A (PKA; Mueller et al., 2008) which enhances neuronal excitability by inhibiting the slow afterhyperpolarization (sAHP; Zhang et al., 2013). However, whether this intracellular signaling cascade underlies retrieval is unknown. Using patch-clamp electrophysiology and the conditioned place preference (CPP) paradigm, we determined whether intrinsic excitability of PL-mPFC neurons maintains memory during retrieval. Rats were conditioned to associate one chamber, but not another, with cocaine. During post-conditioning CPP retention trials, rats had access to both chambers and spent more time in the previously cocaine-paired chamber than in the saline-paired chamber. Microinfusions of the PKA antagonist (RP-2'-O-MB-cAMPs) in the PL-mPFC before the first retrieval test disrupted expression of the CPP on that trial and all subsequent trials. This retrieval deficit was rescued by co-administration of a sAHP antagonist (UCL-2077). *In vitro* patch-clamp recordings were used to examine the physiological consequences of noradrenergic signaling in the PL-mPFC. Pyramidal cells in the PL-mPFC were dialyzed with Rp-cAMPs or not prior to bath application of norepinephrine (NE). In the absence of Rp-cAMPs, NE increased the number of evoked action potentials by reversing the sAHP to a slow afterdepolarization (sADP). However, Rp-cAMPs blocked the NE-induced reversal of the sAHP, and prevented the increase in evoked action potentials. Finally, we examined whether

intrinsic excitability maintains cocaine-associated memory retrieval. Cocaine-conditioned rats were split into low retrieval (LR) and high retrieval (HR) groups based on CPP scores. Patch-clamp recordings from PL-mPFC pyramidal neurons revealed that the number of evoked action potentials was correlated with CPP scores, and was increased in neurons from HR rats compared to LR rats. These data demonstrate that intrinsic neuronal excitability in the PL-mPFC maintains drug-associated memory retrieval through a PKA-dependent signaling cascade. Thus, modulation of the intrinsic excitability of PL-mPFC neurons could have therapeutic potential in the treatment of drug addiction.

Disclosures: **H. Yousuf:** None. **J.M. Otis:** None. **M.K. Fitzgerald:** None. **D. Mueller:** None.

Poster

052. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA038042

University of Wisconsin-Milwaukee Graduate School

Title: The role of medial prefrontal cortex gap junction communication in retrieval and extinction of cocaine seeking

Authors: ***M. FITZGERALD**, J. L. BURKARD, A. GASPARINI, A. ANDERSON, D. MUELLER;
Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: Drug-associated cues can trigger craving and relapse for drug addicts. Preventing retrieval of cue-associated memories or reducing cue reactivity through extinction could reduce relapse rates. Retrieval of a cocaine-associated memory is dependent on activation of the prelimbic medial prefrontal cortex (PL-mPFC; Otis et al., 2013) whereas extinction of cocaine seeking is consolidated in the infralimbic medial prefrontal cortex (IL-mPFC; Otis et al., 2014). Although the neurochemical and synaptic mechanisms underlying drug-related behavior have been investigated extensively, little is known regarding the contribution of gap junction communication between neurons and astrocytes. Both neurons and astrocytes express gap junctions. Gap junctions are specialized membrane structures built of connexin channels that allow cytoplasmic continuity between connected cells. Previous work has demonstrated that

activity of either neuronal or astrocytic gap junctions can alter neuronal activity and plasticity (Palacios-Prado et al., 2014; Pannasch et al., 2011). Thus, we investigated the role of neuronal gap junctions, astrocytic gap junctions, or the combination of both during retrieval and extinction of cocaine seeking using the conditioned place preference (CPP) model. Following conditioning and prior to the first retrieval (15 min) or extinction (30 min) test, rats received a bilateral microinfusion of either a non-selective gap junction blocker (carbenoxolone), a neuron-specific gap junction blocker (quinine), an astrocyte-specific gap junction blocker (IRL-1620), or vehicle into the PL-mPFC or IL-mPFC and were tested daily. General and astrocytic gap junction blockade in the PL-mPFC initially disrupted retrieval of a CPP, but failed to maintain prolonged disruption as demonstrated by a return of CPP in later test trials compared to extinguished controls. Neuronal gap junction blockade in the PL-mPFC enhanced maintenance of the cocaine-associated memory relative to other groups. General, neuronal, or astrocytic gap junction blockade in the IL-mPFC resulted in disrupted extinction learning. These results suggest that individually or collectively disrupting neuronal and astrocytic gap junction networks in the PL-mPFC or IL-mPFC prolong drug-seeking behavior, further suggesting that gap junction communication in the mPFC may play a critical role in drug-seeking behavior.

Disclosures: **M. Fitzgerald:** None. **J.L. Burkard:** None. **A. Gasparini:** None. **A. Anderson:** None. **D. Mueller:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program, NIH.

Title: Identification of distinct neuronal ensembles selectively activated by discrete cues associated with cocaine or heroin seeking in rats

Authors: *F. RUBIO, D. CAPRIOLI, F. SOTO DEL VALLE, M. VENNIRO, V. WALLACE, Y. SHAHAM, B. HOPE;
Behavioral Neurosci. Res. Branch, NIDA IRP, NIH, Baltimore, MD

Abstract: Background: Learned associations between discrete cues (or contexts) and drug effects are thought to be encoded by sparsely distributed patterns of neurons called “neuronal ensembles”. Here, we determined whether cocaine- and heroin-related cues are encoded by

distinct neuronal ensembles within the medial prefrontal cortex (mPFC) in a rat model of drug relapse. Methods: We trained rats to self-administer cocaine (0.75 mg/kg) and heroin (0.0375 mg/kg) on alternate days (3-h/day; 18-days). Cocaine and heroin infusions were paired with two distinct levers and the presentations of discrete cue-lights (20-sec). After 5 days of forced abstinence, rats were assigned to four groups based on the order of two 5 min extinction tests (spaced by 20-min) with the drug-related cues: Cocaine-Cocaine, Heroin-Heroin, Cocaine-Heroin and Heroin-Cocaine. A Non-test control group was trained but kept in the operant chamber on test day. We detected Homer 1a, Arc and Fos mRNA using RNAscope assay. We selected target-probes to detect nuclear signal for Homer 1a- and Arc-activated neurons from the ensembles activated during the first test and second test, respectively. Results: Preliminary analysis showed higher overlap (co-expressing Homer 1a and Arc) in the Cocaine-Cocaine group than in the Heroin-Cocaine, Cocaine-Heroin, and Heroin-Heroin groups for both dorsal and ventral mPFC. Furthermore, 92-98% of these Homer 1a- and Arc-positive cells co-expressed Fos mRNA, a common marker of neural activity. Conclusions: Our results suggest that distinct mPFC neuronal ensembles encode two different drug-cue memories. Further experiments are needed to characterize these ensembles and assess mutually exclusive causal roles in drug-seeking behavior.

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Poster

052. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA034721

NIDA Grant T32 DA07244

Title: Interleukin-1 in the dorsal hippocampus is a novel mediator of acquisition of heroin-conditioned immunosuppression

Authors: ***C. LEBONVILLE**[1], M. E. JONES[1], L. W. HUTSON[1], R. A. FUCHS[2], D. T. LYSLE[1];
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Abstract: Like many other opiates, heroin suppresses the immune system. Similarly, exposure to environments associated with heroin use can produce immunosuppression through Pavlovian conditioning. The dorsal hippocampus (DH) is necessary for this conditioned effect. Interleukin-1β (IL-1β), a pro-inflammatory cytokine expressed in the DH, has been linked to learning and memory. Our laboratory has demonstrated that siRNA-mediated IL-1β gene silencing in the DH prevents the *expression* of heroin-conditioned immunosuppression. Here we investigated the role of DH IL-1 in the *acquisition* of heroin conditioned immunosuppression. To this end, rats were conditioned to associate heroin (1 mg/kg, SC) with a distinct context. During Pavlovian conditioning, rats received bilateral microinfusions of the endogenous IL-1 antagonist (IL-1RA; 1.25 μg/0.5 μL/side) or saline into the DH either 30 min before or 24 h after each of 5 context-heroin pairings. To control for the timing of the injections, each group received saline when the other group received IL-1RA. Six days later, rats were either re-exposed to the heroin-paired context for 60 min or remained in their home cages. Immediately after this manipulation, their immune system was challenged using lipopolysaccharide (LPS, 1 μg/kg, SC), a component of gram negative bacteria. The rats were sacrificed 6 h later. Splenic iNOS, IL-6, IL-1β, and plasma nitrate/nitrite levels were assessed. IL-1RA microinjection 30 min before, but not 24 h after, context-heroin pairing blocked the acquisition of heroin-conditioned immunosuppression, as indicated by no significant suppression of splenic proinflammatory responses to LPS after heroin context re-exposure. Future studies will evaluate whether this time-dependent, IL-1RA-induced acquisition deficit is due to a disruption of associative learning or of the primary immune suppressing effects of heroin during training. Regardless of the mechanism, these findings suggest that elements of the IL-1 signaling pathway may be therapeutic targets for restoring immune function in opiate-exposed populations.

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Poster

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Support: NSERC

CIHR

OGS

Title: Dopamine D3 receptor activity and downstream calcium/calmodulin signaling targets are altered within the basolateral amygdala as a function of opiate exposure state

Authors: *L. G. ROSEN, W. J. RUSHLOW, S. R. LAVIOLETTE;
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Abstract: The potent rewarding effects of opiate drugs facilitate the formation of associative memories linked to the drug-taking experience and play a key role in triggering relapse. These memories are encoded in the basolateral amygdala (BLA). Intra-BLA processing of opiate-related reward memories is mediated by dopamine D1 receptors (D1R) and D2 receptor (D2R) signaling as a function of opiate exposure state. D1Rs are required for acute opiate memory formation in the previously drug-naïve state, but D2R signaling is necessary for memory formation during opiate dependence and withdrawal. The D3 receptor (D3R) is highly expressed in the mesocorticolimbic system, and is also critical for the learning and memory of emotional memory. There is evidence of changes to D3R availability in chronic opiate users, thus raising the question of the molecular mechanisms underlying alterations to D3R expression and function following chronic opiate use. D3R activation is linked to calcium/calmodulin kinase pathways, particularly to cascades involving calcium/calmodulin protein kinase IV (CaMKIV) and calcineurin. These signaling pathways play important roles in synaptic plasticity, and are crucial for the formation of conditioned reward memory in limbic regions. Here, we identify opiate exposure-induced changes to intra-BLA dopamine receptors and their downstream molecular targets. Protein analysis revealed that opiate dependence and withdrawal results in a downregulation of intra-BLA D2R and D3R, but not D1R expression. Additionally, the expression of CaMKIV and calcineurin are profoundly downregulated and upregulated, respectively. We further use a place conditioning procedure paired with targeted microinfusions to probe the behavioural significance of these molecular alterations. We found that intra-BLA D3R activity is not necessary for the formation of opiate reward memories in a previously opiate-naïve state, but that opiate dependence and withdrawal renders these memories sensitive to manipulations of D3R signaling. Ongoing studies are continuing to examine how further manipulations of these molecular pathways may relate to opiate addiction-related memory formation. Overall, this work demonstrates that opiate dependence and withdrawal affects the expression and function of intra-BLA D3Rs and their downstream molecular targets.

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Poster

052. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant R01 DA14498

Title: Remembering to abstain: the impact of working memory on length of first quit attempt in drug users

Authors: *T. E. MOSES[1], E. DUNNE[2], J. J. ROSE[1], W. W. LATIMER[1];
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Abstract: Background: Drug addiction continues to be a major public health problem: more than 20 million Americans aged 12 or older use illicit drugs (NCADD). While much attention is given to treatment and prevention, limited attention is given to the work drug users themselves conduct in order to quit. Most drug users have tried to quit using drugs at some point, and most often find themselves unsuccessful. One factor associated with this difficulty may be related to working memory. Recent research has examined working memory in drug users and found poorer working memory than in non-users. To explore this further, the present study aimed to examine the link between working memory and the length of time an individual was able to abstain from substance use during their first quit attempt. Methods: A secondary data analysis of participants enrolled in the NIDA-funded NEURO II-HIV Prevention RCT study in Baltimore, Maryland. The sample included 167 drug-using adults aged 18 to 59 (M age=42.2, SD=9.8). Participants were community-recruited. They completed an HIV-risk behavior interview that included questions about substance use and whether they had previously attempted to quit using drugs. Participants completed a battery of neuropsychological tests which included the WAIS-III digit span test, which measures forward and backward digit span memory. Linear regression was conducted using the raw scores in order to determine the association between digit span and the length of the time of first quit attempt. Results: Linear regression was conducted to compare digit span total score (M=13.78, SD=4.36) and the length of first quit attempt in years (M=2.28, SD=3.24). Digit span total score was significantly associated with length of first quit attempt, $b = -.22$, $t(165) = -2.88$, $p = .005$, such that those with stronger working memory ability maintained a longer period of nonuse. The results for the Shipley Institute of Living total score (M=33.7, SD=97.20) were included in these analyses to control for general intellectual functioning. Conclusions: Working memory appears to play a role in how long a person is able to maintain abstinence from drugs and alcohol. Individuals who were able to remember more numbers during the digit span test seemed to be able to abstain longer from drugs and alcohol during their first quit attempt than those who remembered fewer. These findings suggest a way in which we can enhance treatment techniques aimed at drug addicts by including memory exercises to improve working memory. Perhaps individuals who are struggling to maintain abstinence may show improved lengths of sobriety--ideally long-term--as working memory is improved.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA09580 (EMU)

Title: PP1/GSK3 signaling pathway is involved in the reconsolidation of cocaine reward memory

Authors: *X. SHI, J. PALMA, E. M. UNTERWALD;
Dept. of Pharmacol. & Ctr. for Substance Abuse Res., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Our previous study demonstrated that glycogen synthase kinase-3 (GSK3) activity is highly induced in nucleus accumbens (NAc), hippocampus, and prefrontal cortex during memory retrieval, and that reconsolidation of cocaine reward memory can be attenuated by inhibition of GSK3. Since protein phosphatase 1 (PP1) is an activator of GSK3β and GSK3β is part of a multi-protein NMDA receptor complex, the roles of PP1 and NMDA receptors in the reconsolidation of cocaine contextual reward memory were investigated in this study. Adult male CD-1 mice underwent cocaine place conditioning for 8 days and were tested for place preference on day 9. Twenty-four hours after the test for place preference, mice were confined to the compartment previous paired with cocaine in a drug-free state for 10 minutes to reactivate cocaine-associated memories. Western blotting indicated that levels of phosphorylated GSK3αSer21 and GSK3βSer9 were down-regulated in the mouse nucleus accumbens and hippocampus after the reactivation of cocaine cue memories, consistent with our previous findings. Interestingly, PP1 inhibition with okadaic acid (OA, 150 ng/3ul, i.c.v) 30 minutes before re-exposure to the compartment previously paired with cocaine prevented the decrease in phosphorylated GSK3α/β in both nucleus accumbens and hippocampus. Furthermore, administration of OA 30 minutes prior to the reactivation of cocaine cue memories abrogated a previously established place preference when tested 24 hours later. Similarly, administration of the NMDA receptor antagonist MK-801 (0.3 mg/kg, i.p.) immediately after re-exposure to cocaine-paired compartment disrupted the previously established place preference, suggesting interference with reconsolidation of cocaine-associated reward memories. The role of nucleus accumbens in the reconsolidation of cocaine associated memories was also investigated in this study. Immediately after the reactivation session, microinjection of SB216763, a selective inhibitor of GSK-3, into nucleus accumbens blocked the reconsolidation of cocaine-associated

reward memories. These findings suggest that the dephosphorylation of GSK3 that occurred upon activation of cocaine-associated reward memory may be initiated by the activation of PP1 during the induction of NMDA receptor-dependent reconsolidation of cocaine-related memory. Moreover, the role of PP1 and NMDA receptor in cocaine memory reconsolidation makes them potential therapeutic targets in treatment of cocaine addiction and prevention of relapse.

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Poster

052. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: Australian Postgraduate Award (APA) to MC

UNSW Faculty of Science Research Grant

Title: Transcriptional and epigenetic factors underlying the extinction of nicotine-seeking behaviour in the rat

Authors: *M. R. CASTINO[1], N. A. YOUNGSON[2], D. BAKER-ANDRESEN[3], V. S. RATNU[3], T. W. BREDY[3,4], K. J. CLEMENS[1];
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Abstract: Relapse to cigarette smoking is a principle characteristic of tobacco addiction. This may be due to the persistence of drug-associated memories that prompt drug use across abstinence. This clinical finding can be modelled in rodents, as rats previously trained to self-administer nicotine are susceptible to reinstatement of drug-seeking following exposure to priming doses of nicotine, or cues previously associated with its delivery. Like other forms of memory, drug-associated memories appear to depend on changes in gene expression that are coordinated, in part, by epigenetic mechanisms. Research in this field suggests that the formation and extinction of contextual drug memories are regulated by dynamic modifications to chromatin. Previous work in our laboratory has demonstrated that administration of the histone deacetylase (HDAC) inhibitor, sodium butyrate (NaB), facilitates the extinction of nicotine-

seeking in a manner that provides resistance to reinstatement. The present study aimed to investigate the molecular mechanisms involved in this potentiated extinction learning, examining the effect of both nicotine exposure and NaB treatment on mRNA expression and histone acetylation in the rat medial prefrontal cortex. Sodium butyrate induced a significant increase in gene expression of the neuronal protein kinase, Cdk5, in both saline and nicotine treated rats. In contrast, NaB increased BDNF mRNA only in saline control rats. Furthermore, nicotine, but not saline, exposure induced a decrease in histone H3K14 acetylation at the BDNF Exon IV promoter that was normalised by NaB treatment. These findings indicate that NaB and nicotine may act individually and in concert to regulate the genetic profile of the brain, though the behavioural consequences of their interactions are yet to be fully elucidated.

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Poster

052. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: This research was supported financially by the Psychology Department at Dickinson College.

Title: Timing of SCH 23390 administration influences extinction of conditioned hyperactivity in mice

Authors: *A. S. RAUHUT[1,2], K. A. RATNER[2], S. BUCK[2], E.-R. SUNG[2];
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Abstract: Previous research has suggested that the dopamine subtype-1 (D1) receptor system has a role in extinction of context-drug memories. However, the precise role of the D1 receptor system in different memory processes (i.e., retrieval vs. reconsolidation) involved with extinction of conditioned hyperactivity is unknown. Thus, the present experiments determined the effect of a selective D1 receptor antagonist, SCH 23390, on extinction of conditioned hyperactivity produced by methamphetamine when SCH 23390 was administered immediately after (memory reconsolidation; Experiment 1), or administered before (memory retrieval; Experiment 2), daily extinction sessions. The experiments consisted of 2 phases: acquisition and extinction. During the acquisition phase, male, Swiss-Webster mice received injections (s.c.) of either vehicle

(physiological saline; unpaired) or methamphetamine (1.0 mg/kg; paired) immediately prior to 30-minute sessions in the locomotor activity chambers on Chamber Days 1, 3, 5 and 7. On the intervening days (Chamber Days 2, 4, 6 and 8), paired and unpaired mice received injections of either vehicle or methamphetamine in their home cages, respectively. Following the acquisition phase, paired and unpaired mice received daily injections of vehicle immediately prior to 30-minute sessions in the locomotor activity chambers for 5 (Experiment 1) or 7 (Experiment 2) consecutive sessions (extinction). Paired and unpaired mice also received daily injections (i.p.) of either vehicle (physiological saline) or SCH 23390 (0.0125, 0.025, 0.05 mg/kg) immediately after (Experiment 1), or received daily injections of vehicle or SCH 23390 (0.05 mg/kg) 30 minutes before (Experiment 2), the extinction sessions. In both experiments, methamphetamine produced robust conditioned hyperactivity followed by extinction. Furthermore, we found that SCH 23390 (0.05 mg/kg) facilitated the rate of extinction when administered prior to the session, but did not alter the rate of extinction when administered immediately following the sessions. Taken together, these results suggest that the D1 receptor is involved with memory retrieval (or expression), but not memory reconsolidation, processes during extinction of conditioned hyperactivity.

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Poster

052. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIAAA R37AA011852

Title: Modeling Pavlovian alcohol seeking in rats using a retractable sipper to study both appetitive and consummatory behavior

Authors: *R. U. COFRESI[1], S. M. LEWIS[1], N. CHAUDHRI[2], H. J. LEE[1], M. H. MONFILS[1], R. A. GONZALES[1];
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Abstract: Appetitive conditioned responses elicited by cues that predict access to alcohol may promote problematic alcohol use in individuals at varying risk for alcohol abuse and alcoholism. We developed an appetitive Pavlovian paradigm in which time-limited access to unsweetened alcohol (10% or 15% v/v ethanol in tap water: 10E or 15E) via a retractable sipper tube and

bottle assembly served as the reinforcer. In our paradigm, 10s after the houselight turned on, the sipper inserted into the chamber, and after 10s elapsed, the sipper retracted, and the houselight turned off. Several such trials were given per conditioning session, with 4 min elapsing between trials on average. All sessions were recorded and all trials were scored for behaviors of interest (rearing/orienting, sipper site approach, sipper contact) using a sampling method. Lickometers provided an additional, direct measurement of sipper contact. We first induced drinking in 12 singly housed, adult, male Long-Evans rats by providing 24 hr access to unsweetened alcohol in the homecage on MWF for 5 weeks (standard chow and water were available ad libitum; mean ± s.e.m. ethanol intake over last 3 sessions drinking 10E or 15E = 3.2±0.4 g/kg). Rats were then conditioned over 12 consecutive daily sessions, each providing 8 light-sipper pairings. Despite only 8 access periods per session, each only 10s long, rats ingested psychopharmacologically relevant quantities of ethanol (mean ± s.e.m. ethanol intake over last 3 sessions drinking 10E or 15E = 0.58 ±0 .07 g/kg; mean ± s.e.m. blood ethanol content = 22±7 mg/dL 5-10 min post-session). Two cue-conditioned responses developed in all rats: approach to the sipper site during the 5s prior to sipper insertion and sipper contact upon presentation. Conditioned approach and contact were then extinguished over 12-14 consecutive daily sessions, each providing 12 light-sipper pairings with an empty bottle. A long-term memory test (4 light-sipper pairings, empty bottle) conducted 48 hr after the last extinction session revealed low spontaneous recovery of conditioned responses. Providing the scent of alcohol during a second memory test conducted 48 hr later resulted in reinstatement of conditioned sipper approach ($F_{1,11}$=7.118, p=0.0219) and contact ($F_{1,11}$=13.77, p=0.003) relative to the first memory test. In conclusion, we established a model of Pavlovian alcohol seeking in adult, male rats. In traditional preparations, fluid and food ports allow measurement of only appetitive behavior. Importantly, the use of a retractable sipper in our preparation allows us to measure both appetitive and consummatory behavior.

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Poster

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Support: NIH Grant DA012844

NIH Grant DA026356

Title: Nicotine attenuates the effects of HIV-1 proteins on the neural circuitry of working and contextual memory

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Abstract: Human immunodeficiency virus (HIV)-1-associated neurocognitive disorders (HAND) are characterized by synaptic damage and neuronal loss in the brain. Excessive glutamatergic transmission and loss of cholinergic neurons are the major indicators of HAND. By acting as a cholinergic channel modulator, the cognitive-enhancing effect of nicotine in neurodegenerative and cognitive disorders has been documented. However, it remains to be determined whether nicotine has any beneficial effects on memory and synaptic plasticity formation in HAND. In this study, we investigated the effects of nicotine on synaptic plasticity and hippocampus/prefrontal cortex (PFC)/amygdala-dependent memory formation in HIV-1Tg and F344 control rats. Chronic nicotine treatment (0.4 mg/kg/day, s.c.) significantly attenuated the cognitive deficits in both spatial and contextual fear memory in the HIV-1Tg rats, but impaired the contextual learning memory in the F344 rats. To further determine the role of nicotine in the synaptic dysfunction caused by HIV-1 proteins, we analyzed the expression of key representative genes related to synaptic plasticity in the hippocampus, PFC, and amygdala of HIV-1Tg and F344 rats using a custom-designed qRT-PCR array. The HIV-1 proteins significantly altered the glutamate receptor-mediated intracellular calcium cascade and its downstream signaling cascade in a brain region-specific manner. Further, chronic nicotine treatment reversed the effects of the HIV-1 proteins on the expression of genes involved in synaptic plasticity in the three brain regions. The effects of nicotine differed significantly in the HIV-1Tg and F344 rats. Our findings indicate that nicotine can attenuate the effects of viral proteins on cognitive function and produce brain region- and strain-specific effects on the intracellular signaling cascades involved in synaptic plasticity and memory formation.
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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: 1057/MOB/2013/0

Title: Common brain mechanism underlying pathological gambling and problematic pornography consumption

Authors: *M. K. GOLA[1,2], M. WORDECHA[3], G. SESCOUSSE[4], B. KOSSOWSKI[3], M. WYPYCH[3], M. LEW-STAROWICZ[5], A. MARCHEWKA[3];
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Abstract: In recent years clinicians have observed an increase in the number of people suffering from out-of-control sexual behaviors (OCSB) such as excessive pornography consumption, masturbation or use of paid sexual services. There is ongoing discussion on how to conceptualize these behaviors and perform efficient psychotherapeutic or pharmacological intervention. To address this issue we have used an incentive delay task that was previously employed to study pathological gambling (PG) - a well-characterized behavioral addiction. This previous study (Sescousse et al., 2013) showed that pathological gamblers are characterized by a blunted VStr response to non-monetary reward cues. The magnitude of this effect was correlated with gambling severity and accompanied by lower motivation for erotic cues. If the brain mechanisms underlying OCSB are similar to PG, we should expect the opposite pattern, i.e. a blunted VStr response to non-erotic cues, correlated with OCSB severity and diminished motivation. We examined this hypothesis in 28 Caucasian heterosexual males. Half of them were in treatment because of excessive pornography consumption and masturbation. This 'OCSB group' met the criteria of hypersexual disorder5 (HD; Kafka, 2010) and had no other psychiatric conditions (according to ICD 10). The other 14 participants were pornography users who did not qualify for HD and were matched on age and income. We used fMRI and the exact same protocol as the in previous study on PG (Sescousse, et al., 2013) In line with our prediction there was a significant interaction between group and monetary/erotic cues on VStr reactivity, primarily driven by a diminished VStr response to non-erotic (i.e. monetary) cues in OCSB individuals. The differential VStr reactivity to erotic and monetary cues was correlated with the relative motivation for these two rewards (as measured by RTs) as well as with OCSB symptoms (as measured by the Sexual Addiction Screening Test) and frequency of masturbation. Given the role of the VStr in reward learning and expectation (Berridge et al., 2009), these results suggest that in individuals with OCSB, erotic cues may act as powerful incentives overriding the motivational value of alternative sources of reward (such as money) and more likely drive them to out-of-control behaviors. Our findings points also to a possible overlap between OCSB and the concept of behavioral addiction.

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Poster

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Title: Acute methamphetamine produces long-term deficits in hippocampal-dependent spatial learning and memory retention, decreases PKMzeta, GluA2 and dopamine 1 receptors (D1), and increases microglial expression

Authors: *S. BRAREN[1], D. DRAPALA[1], J. AVILA[1,2], P. A. SERRANO[1,2];
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Abstract: The purpose of our study was to evaluate the effects of two bolus doses of methamphetamine (MA; 30 mg/kg) on spatial learning and memory. MA is known to have significant effects on dopaminergic terminals within the striatum and also known to decrease Dopamine 1 receptor (D1) expression in the hippocampus (Braren, 2014 Front. Behav. Neuro. 4, 438). We evaluated spatial learning and memory using the hippocampal dependent task, the radial 8-arm maze (RAM). The hippocampi from this study were evaluated for the expression of several synaptic markers important for memory and synaptic plasticity. These include the D1 receptor, the atypical protein kinase M zeta (PKMζ), and the AMPA receptor subunit GluA2. PKMζ is important for trafficking the GluA2 subunit to the membrane and maintaining GluA2 on the membrane improves memory retention (Migues, 2010 Nat. Neurosci. 13, 630-4; Sebastian, 2013 PLoS One. 8, e81121). We hypothesize that neuroinflammation plays a role in exacerbating the negative effects of MA-induced memory deficits. One inflammatory marker in particular, COX2, is one of two isoenzymes, which catalyze the conversion of arachidonic acid into prostaglandins. Several prostaglandins are catalyzed by COX2, but one in particular is very toxic, the prostaglandin J2 (PGJ2). We have found that PGJ2 can induce memory deficits when

injected into the hippocampus. Thus, we are interested in determining how MA toxicity may activate this inflammatory pathway involving PGJ2, perpetuating the toxicity leading to sustained cognitive deficits. Our results show that two bolus doses of MA delivered 1 week apart produces deficits in learning the RAM 7 weeks later and decreases PKMζ, GluA2 and D1 expression. These results identify the long lasting effects of MA on hippocampal function. Additionally, delivering these two bolus doses of MA after acquiring the RAM memory results in retrieval deficits at 2 weeks after the end of training and results in increased in microglia expression in the CA1 region of the hippocampus. These results elucidate the long lasting learning and memory impairments from acute MA treatment. We hypothesize that these long-term effects of MA are mediated through an inflammatory pathway, as microglia expression is upregulated after MA. Future studies will examine the role of COX2 expression in these samples.

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Poster

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Title: Activation and transition of the ventral and dorsal striatum during cue reactivity in Internet gaming disorder

Authors: ***L. LIU**[1], J.-T. ZHANG[2], L.-J. WANG[2], B. LIU[2], S.-S. MA[2], Y.-W. YAO[2], X.-Y. FANG[1];
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Abstract: According to dual-systems accounts, instrumental behavior consists of two dissociable learning processes mainly mediated by corticostriatal pathways: goal-directed system is performed according to the relationship between actions and their consequences mediated by VMPFC, ventral striatum (VS); habitual system controlled by dorsal striatum (DS) is directly

triggered by related cues even when the outcomes have lost their goal values. As a potent forms of reinforcement learning, many studies have focused on substance dependence and supported the hypothesis that drug addiction can be viewed as the endpoint of a series of transitions from initial voluntarily drug use to habitual drug use. And the addiction is the result of dynamic shifts in the neural control over behavior, from ventral to dorsal striatal control. But this transition hasn't previously been studied in Internet Gaming Disorder (IGD), which has been identified in Section III of the DSM-V as a non-substance addiction requiring further study. **Methods** We used a cue-reactivity functional magnetic resonance imaging (fMRI) design during which pictures of Internet games and general Internet surfing stimuli were presented. Thirty-nine IGDs (18.92±9.85 gaming hours/ week, score of Chinese Internet Addiction Scale (CIAS) > 67) and 23 matched healthy controls (HC) (0.92±0.20 gaming hours/ week, score of CIAS < 60) were examined. **Results** IGDs showed significant higher activations in the VS and DS compared to HC [contrast 'game > general Internet cues'; region of interest analyses: $P < 0.05$ false discovery rate-corrected]. In IGDs, the left VS and right caudate activation correlated negatively with self-reported cue-induced craving (Pearson's $r = -0.34$, $P = 0.03$; $r = -0.31$, $P = 0.05$). Positive associations were found between cue-induced activation in the DS (right pallidum and putamen) and duration of current online gaming state in IGDs ($r = 0.43$, $P = 0.007$; $r = 0.29$, $P = 0.07$). Furthermore, cue-induced activation in the putamen was negatively associated with VS volume ($r = - 0.33$, $P = 0.04$). **Conclusions** In line with the hypothesis we found higher cue-induced activation of the VS and DS in IGDs, and the decreasing relevance of cue processing in the ventral and dorsomedial part of striatum involved goal-directed actions, whereas the increasing involvement of gamer's DS in cue processing. Furthermore the aberrant of VS morphometry may underlie the dominant of DS, which may implicate the deficit of goal-directed processing and the over-reliance on habitual system. Our results excluded the impact of substance on dopamine functioning, suggesting that the transition may also be a general feature of behavioral addiction.

Disclosures: **L. Liu:** None. **J. Zhang:** None. **L. Wang:** None. **B. Liu:** None. **S. Ma:** None. **Y. Yao:** None. **X. Fang:** None.

Poster

052. Learning, Memory, Dependence, and Addiction

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 52.17/G36

Topic: C.17. Drugs of Abuse and Addiction

Support: Fundación Sociosanitaria de Castilla-La Mancha Grant PI2009/36

Title: High fat dieting delays the extinction of conditioned preference for places paired to palatable food and upregulates addiction biomarkers in the nucleus accumbens

Authors: ***J. M. PÉREZ-ORTIZ**, A. GALIANA, E. SALAS, C. GONZÁLEZ-MARTÍN, M. GARCÍA-ROJO, L. F. ALGUACIL;
Translational Res. Unit, Univ. Gen. Hosp. of Ciudad Real, Ciudad Real, Spain

Abstract: We have developed a mouse model of food addiction to quantify the effect of dietary fat on palatable food seeking and to validate three proteins as potential biomarkers of addiction vulnerability in the nucleus accumbens: fumarate hydratase (FH), ATP synthase subunit alpha (ATP5a1) and transketolase (TKT). Forty C57BL/6J male mice, four-weeks old, were fed either with a high fat diet (HF) or a standard diet (controls). After two weeks animals started a daily training of double conditioning sessions; in one of them animals were placed in a compartment with palatable food (chocolate cereals), while in the other mice were placed in a different compartment with no food at all. After one week this training was reduced to one daily session preceded by 12 h of food deprivation. A final session was conducted one week later that consisted in placing half of the animals in food unpaired compartments and the other half in compartments previously paired with cereals, but now devoid of the expected palatable food. Animal activity was recorded by videotracking. When mice were placed in compartments devoid of palatable food they initially spent most of their time in the small area around the feeder (% time 0-10 min: controls = 53 ± 4, n = 10; HF = 54 ± 5, n =10). Control mice gradually extinguished this behavior, which was more persistent in HF mice (% time 20-30 min: controls = 39 ± 4, $p < 0.05$ vs 0-10 min; HF = 49 ± 7). Total activity and serum corticosterone levels were similar in all groups regardless the diet or compartment considered. Immunohistochemistry of FH, ATP5a1 and TKT showed an increase in the expression level of the three markers in the group of HF mice. Moreover, a positive correlation was found between those expression levels and the persistence of preference for the palatable-food feeder in HF and standard fed mice, as quantified by the time spent in the food area 20-30 min after the start of the behavioral test. The procedure seemed useful to quantify food addiction as delayed extinction of preference for places paired with palatable foods, and confirmed FH, ATP5a1 and TKT expression in the nucleus accumbens as correlates for drug and food addiction.

Disclosures: **J.M. Pérez-Ortiz:** None. **A. Galiana:** None. **E. Salas:** None. **C. González-Martín:** None. **M. García-Rojo:** None. **L.F. Alguacil:** None.

Poster

052. Learning, Memory, Dependence, and Addiction

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 52.18/G37

Topic: C.17. Drugs of Abuse and Addiction

Support: DA017949

Title: Chronic fluoxetine ameliorates long-term trace conditioning deficits in mice exposed to chronic nicotine during adolescence

Authors: *D. A. CONNOR[1], T. J. GOULD[2];
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Abstract: Adolescence represents a critical period in which brain areas important for cognition, including the hippocampus and medial prefrontal cortex (mPFC), rapidly develop. As a result, adolescence marks a time when the CNS is sensitive to exogenous insults. Exposure to chronic nicotine during adolescence causes long-term changes in synaptic function, which disrupts cognition. In addition, adolescent nicotine exposure results in cognitive deficits later in life, i.e., in adulthood. In 2014, the CDC reported that 24% of high school students used tobacco products. Given that cognitive deficits are associated with smoking relapse and addiction, long-term cognitive deficits that result from adolescent nicotine exposure may lead to increased risk of nicotine dependence in adulthood. Indeed, adolescent exposure to tobacco increases risk for adult smoking by a factor of 16. Thus, it is important to investigate treatments that ameliorate the long-term cognitive impacts of adolescent nicotine exposure. Here we investigated whether or not chronic fluoxetine (FLX) could ameliorate adolescent chronic nicotine-associated disruption of trace fear conditioning. Trace fear conditioning is a hippocampus-depended form of associative learning well suited to investigate adolescent nicotine-associated cognitive deficits as it also recruits the mPFC via working memory processes. Chronic FLX was selected as a treatment due to its ability to increase BDNF within the mPFC and hippocampus, as well work showing it can reduce hippocampus-dependent cognitive impairments. Adolescent (PND 38) C57BL/6J mice were administered chronic nicotine or saline via osmotic minipumps for 12 days. Upon pump removal mice were immediately put on FLX treatment via their drinking water (160g/L). After 30 days of FLX treatment, these same mice, now adult aged, were trained in trace fear conditioning and tested 24 hr later with FLX treatment maintained throughout. Mice that received chronic nicotine treatment during adolescence demonstrate deficits in trace fear conditioning during adulthood, while mice that received saline did not. In addition, chronic FLX ameliorated the nicotine-associated deficit in trace fear conditioning. Our data support previous findings that nicotine administration during adolescence leads to deficits in hippocampus-dependent learning. Furthermore, our data suggest that chronic fluoxetine treatment may be protective against development of long-term cognitive deficits associated with adolescent nicotine exposure. In sum, FLX may be an effective therapy for long-term cognitive deficits associated with adolescent nicotine exposure.

Disclosures: D.A. Connor: None. **T.J. Gould:** None.

Poster

052. Learning, Memory, Dependence, and Addiction

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 52.19/G38

Topic: C.17. Drugs of Abuse and Addiction

Support: Start-up funds-Kansas State University

Title: Individual differences in voluntary alcohol consumption predict reversal learning performance in rats

Authors: M. GALLO, N. BRIGHT, H. FISHER, M. GREER, A. PAJSER, M. RAY, M. WANG, *C. L. PICKENS;
Kansas State Univ., Manhattan, KS

Abstract: Alcohol abuse is associated with impaired decision-making. However, it is unclear whether alcohol consumption causes impaired decision-making, or if alcohol consumption and poor decision-making result from pre-existing tendencies that are present before alcohol abuse. The control provided by animal models can address this question in ways that human research cannot. Our study investigated whether the motivation to consume alcohol correlates with reversal-learning performance (a model of decision-making) in rats. The rats received 6 weeks of chronic intermittent access to alcohol beginning in adolescence and continuing on until early adulthood (PND 26-66). Half of the rats received 24-h alcohol access periods 3 times per week where 20% alcohol was available (Alcohol group). The control group received only water during the 6 weeks (Water group). Seventeen days after the final access period, rats began go/no-go discrimination training in which rats earned a food pellet for pressing one lever and earned a pellet for withholding responding from another lever. Once the rats met the discrimination criterion, reversal training began. In the first reversal, the rats were required to press the lever that previously required withholding responding and to withhold lever pressing to the lever they were previously required to press. Once the rats attained the reversal criterion, the contingencies were reversed back to the original contingencies. We found no difference between the Alcohol vs. Water group during any phase. However, there was a significant negative correlation between the quantity of alcohol consumed during the final two weeks of alcohol access and the number of commission errors within the Alcohol group in both reversals. There was no correlation found between the quantity of alcohol consumed and the number of commission errors in the original discrimination, or the number of omission errors during any phase. Our results suggest that rats that consume higher levels of alcohol in early adulthood exhibit better response inhibition in a reversal learning task. We found no evidence that the consumption of alcohol impaired reversal

learning or response inhibition. This suggests that individual differences in motivation to consume alcohol are correlated with performance in a decision-making task, apart from any direct effects of alcohol consumption on the decision-making brain circuits. Our future studies in the lab will investigate potential genetic differences or neural mechanisms in the brain, as well as potential sex differences in the pattern of reversal learning performance.

Disclosures: **M. Gallo:** None. **N. Bright:** None. **H. Fisher:** None. **M. Greer:** None. **A. Pajser:** None. **M. Ray:** None. **M. Wang:** None. **C.L. Pickens:** None.

Poster

052. Learning, Memory, Dependence, and Addiction

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Program#/Poster#: 52.20/G39

Topic: C.17. Drugs of Abuse and Addiction

Support: NRF-2013R1A1A2065207

NRF-2014M3C7A1062893

Title: The neural responses of cognitive performance deficits in Internet gaming disorder during prospective memory task

Authors: *J. CHUN, J. KIM, A. PYEON, H. CHO, J. CHOI, D. KIM;
Dept. of Psychiatry, The Catholic Univ. of Korea Col. of Medici, Seoul, Korea, Republic of

Abstract: As Internet Gaming Disorder (IGD) was added to Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), the seriousness of IGD was magnified recently. The aim of this study was to investigate cognitive performance deficits in Internet gaming disorder during prospective memory task by assessing behavior task results between IGD group and healthy control (HC) group playing Internet game. Seventeen with IGD and fifteen HC were recruited for the fMRI study and underwent the structured interview by a clinician. All participants had normal or corrected-to-normal vision and were right-handed (assessed by the Edinburgh handedness inventory). The participants had to decide congruency (duration 2,000 ms) of shape or color in two figures (figure matching task) as a foreground task. While the task was ongoing, the participants performed prospective memory task and they had to remember a prospective memory trial number presented in the upper right corner of the screen. Functional and structural MRI data were acquired using a 3T MRI system equipped with an 8-channel head coil. Participants' heads were cushioned with attached earmuffs. The functional images were

obtained using a T2*-weighted gradient echo-planar imaging sequence (38 slices, 4 mm thickness and no gaps, repetition time [TR] = 2,000 ms, echo time [TE] = 30 ms, flip angle [FA] = 90°, image matrix = 64 x 64, field of view [FOV] = 220 mm, with voxel resolution of 3.75 x 3.75 x 3.85 mm. Structural MRI data were also acquired using a magnetization-prepared rapid gradient echo sequence: TR = 1.780 ms, TE = 2.19 ms, voxel size = 0.50 mm × 0.50 mm × 1.00 mm, matrix size = 512 × 512, and slice number = 176. In the behavioral results, IGD group compared with HC group showed lower accuracy and slower reaction time on long-term prospective memory condition. In the fMRI results, IGD group compared with HC showed deactivation on the dorsal lateral prefrontal cortex and visual cortex during short-term prospective memory condition. Also, IGD group compared with HC revealed deactivation on the medial prefrontal cortex including anterior cingulate cortex and orbito-prefrontal cortex. In this study, we investigated cognitive performance when remembering to perform a planned action in IGD using prospective memory task. Participants with IGD revealed deficit of performance when they had to perform a planned action during prospective memory and they showed neural deactivation the regions related to cognitive control. It suggested that a planned action induced cognitive conflict in IGD and they showed cognitive deficit.

Disclosures: **J. Chun:** None. **J. Kim:** None. **A. Pyeon:** None. **H. Cho:** None. **J. Choi:** None. **D. Kim:** None.

Poster

052. Learning, Memory, Dependence, and Addiction

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 52.21/G40

Topic: C.17. Drugs of Abuse and Addiction

Support: TRDRP Award #21RT-0136

Title: Age and dose are important factors in the acquisition phase of the tobacco dependence animal model

Authors: *C. GELLNER;
Pharmacol., Univ. of California, Irvine, Irvine, CA

Abstract: To understand how factors such as age, dose, and type of drug tested affect the acquisition phase of the tobacco dependence animal model, we have established a model of intravenous self-administration (IVSA) comparing cigarette smoke extract (CSE) to nicotine (Nic) alone at five different doses in adolescent and adult male rats. 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 (aged postnatal day 25, P25) and adult (P85) rats were trained to work for food pellets on an FR1TO20 schedule (one food pellet per lever press with a time out period of 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, adolescents and adults, respectively) underwent three progressively harder schedules of lever pressing: FR1TO20, FR2TO20, and FR5TO20 for one of 5 doses of CSE or Nic (0, 3.75, 7.5, 15, or 30µg/kg/infusion nicotine content). We have found that both adolescent and adult rats acquire self-administration behavior of CSE or Nic at all doses. Although there are no differences between CSE and Nic reinforcement at either age, adolescents take more drug than their adult counterparts at all doses on an FR1 schedule and at the 30µg/kg/infusion dose of nicotine on an FR5 schedule. These findings suggest that age and dose are important factors in acquisition phase of the tobacco dependence animal model.

Disclosures: C. Gellner: None.

Poster

052. Learning, Memory, Dependence, and Addiction

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 52.22/G41

Topic: C.17. Drugs of Abuse and Addiction

Title: The effects of chronic Cigarette Smoke Extract exposure on somatic withdrawal in adolescent rats

Authors: *D. REYNAGA, A. REZK, C. PON, D. GHOBRIAL, F. LESLIE;
Univ. of California Irvine, Irvine, CA

Abstract: Adolescence is a sensitive period of development where the initiation of smoking typically occurs. Human adolescents are especially sensitive to withdrawal, exhibiting symptoms of dependence soon after smoking initiation and before the formation of daily smoking habits. In adult rodents, discontinuation of chronic nicotine administration results in the development of many of the same characteristic withdrawal symptoms as humans. However, whereas human adolescents have an increased sensitivity to the effects of smoking cessation, rodent adolescents experience little to no somatic withdrawal symptoms after chronic nicotine exposure (O'Dell,

2009; Torres et al., 2013). This discrepancy may be due to the lack of other non-nicotine smoke constituents in current preclinical models. We have previously shown that cessation to chronic exposure of cigarette smoke extract (CSE), a saline solution containing nicotine and other aqueous smoke constituents collected from whole stream cigarette smoke, resulted in enhanced spontaneous somatic withdrawal in adult rats. The goal of this study is to determine whether adolescent rats show spontaneous somatic withdrawal symptoms after chronic exposure to CSE, and to investigate the involvement of nicotinic acetylcholine receptors (nAChRs) in CSE cessation-induced withdrawal using mecamylamine, a nonselective and noncompetitive nAChR antagonist, to precipitate withdrawal. Adolescent rats were treated by intravenous administration of saline, nicotine (1.5 mg/kg/day), or CSE (1.5 mg/kg/day nicotine content) 3 times a day for 10 consecutive days. Following the last day of drug treatment, spontaneous or precipitated somatic signs of withdrawal (gasps, writhes, ptosis, body shakes, teeth chattering, chewing, yawns, and shortness of breath) were scored. Adolescent rats showed spontaneous somatic withdrawal after CSE exposure, but not after chronic nicotine. Mecamylamine (1 mg/kg; s.c.) administration precipitated withdrawal after chronic CSE exposure. This provides evidence that non-nicotine constituents in CSE mediate withdrawal processes, possibly via nAChRs .

Disclosures: **D. Reynaga:** None. **A. Rezk:** None. **C. Pon:** None. **D. Ghobrial:** None. **F. Leslie:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.19. Drug Discovery and Development

Support: NIH Common Fund

NIH Intramural Research Program of TRND/NCATS and CRM/NIAMS, NIH

Title: A high-throughput phenotypic screen to identify compounds that protect human astrocytes from oxidative stress: a proof-of-concept study using stem cell-derived astrocytes for neurodegenerative disease drug discovery

Authors: ***N. THORNE**[1], N. MALIK[2], S. SHAH[2], J. ZHAO[1], B. CLASS[1], F. AGUISANDA[1], N. SOUTHALL[1], M. XIA[1], M. RAO[3], W. ZHENG[1];
[1]NCATS/NIH, Rockville, MD; [2]NIAMS/NIH, Bethesda, MD; [3]NIH Ctr. for Regenerative Med., Bethesda, MD

Abstract: It is becoming increasingly clear that astrocytes play key roles in proper neuronal function in the central nervous system. Astrocytes serve to maintain an extracellular environment conducive to neuronal signaling, in addition to supplying neurons with nutrients necessary for cell health and metabolic precursors that can be used for neurotransmitter synthesis. Recently, astrocytes have been implicated in the pathogenesis of a variety of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS), and have thus become a new target for drug discovery. In order to identify potential therapeutics for these diseases, we have developed astrocyte-based assays that can be used for high-throughput screening (HTS), and that can be used to screen astrocytes differentiated from induced pluripotent stem cells (iPSC) from neurodegenerative disease patients. Towards this effort, our group has developed methods for successfully growing and maintaining astrocytes differentiated from human embryonic stem cells (hESCs) in 1536-well microtiter plate format. Additionally, we have developed a high-content screening assay in 1536-well format to identify compounds that are cytoprotective to astrocytes challenged with oxidative conditions – conditions characteristic of neurodegenerative disease. Using this screening assay, we identified 32 compounds that prevented apoptosis in astrocytes faced with oxidative stress. Some of these compounds were found to activate the antioxidant response element (ARE) and Nrf2 (nuclear factor erythroid 2 [NF-E2]-related factor 2) pathway, while others have previously been shown to be neuroprotective. This miniaturized oxidative stress assay can thus be successfully used to screen chemical libraries using patient-derived astrocytes to identify lead compounds for drug development.

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Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.02/G43

Topic: C.19. Drug Discovery and Development

Support: 2014CB942804

31271123

Title: Minocycline inhibits inflammatory responses but does not prevent neuronal loss in a mouse model of neurodegeneration

Authors: *G. CHEN, S. CHENG, J. HOU, C. ZHANG;
Nanjing Univ., JIANGSU, China

Abstract: Accumulating evidence has shown that minocycline, a broad-spectrum tetracycline antibiotic, exhibits neuroprotective effects in various animal models of neurological diseases. However, it remained unknown whether minocycline could be used effectively to prevent neurodegenerative diseases. To address this question, minocycline was used to treat Dicer conditional knockout (cKO) mice which display age-related neuron loss. The drug was given to mutant mice prior to the occurrence of neuroinflammation, and the treatment had lasted 2 months. Levels of inflammatory markers, including glial fibrillary acidic protein (GFAP), ionized calcium-binding adapter molecule1 (Iba1) and interleukin6 (IL6), were significantly reduced in minocycline-treated Dicer cKO mice. In contrast, levels of neuronal markers and the total number of apoptotic cells in Dicer cKO mice were not affected by the drug. Therefore, minocycline is effective to inhibit neuroinflammation but does not prevent neurodegeneration.

Disclosures: **G. Chen:** None. **S. Cheng:** None. **J. Hou:** None. **C. Zhang:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

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Program#/Poster#: 53.03/G44

Topic: C.19. Drug Discovery and Development

Support: HIH grant 1R41AG044024-01A1

Title: cGMP/CREB signaling as therapeutic target for the chronic or acute treatment of Alzheimer’s disease, migraine and epilepsy

Authors: *M. BEN AISSA[1], R. P. GANDHI[1], S. H. LEE[1], A. PRADHAN[2], I. GAISINA[1], G. R. THATCHER[1];
[1]Dept. of Medicinal Chem. and Pharmacognos, Chicago, IL; [2]Dept. of Psychiatry, Col. of medecine, UIC, Chicago, IL

Abstract: cGMP/CREB signaling is involved in several stimulus-induced cellular signaling pathways leading to the transcriptional control of numerous genes implicated in many important physiological functions in the CNS. Depression of the CREB pathway is a feature of Alzheimer's disease (AD) associated with synaptic dysfunction and coupled with amyloidogenesis. Conversely, aberrant activation of NO/cGMP/CREB signaling has been related to migraine

induced hyperalgesia and persistent MAPK/CREB-mediated gene transcription associated with discrete brain insults has been proposed to underlie epileptogenesis. The overarching objective is to discover the therapeutic potential of brain bioavailable inhibitors and activators of cGMP formation, requiring validation of both *in vitro* and *in vivo* models. For AD therapeutic intervention, a new cGMP/CREB activator NMZ was validated in three FAD mouse models APP/PS1, 3xTg and EFAD and in a new AD sporadic model. NMZ treatment attenuated hallmark AD pathology and restored LTP and memory as well as biomarkers of synaptic and neuronal functions. A second activator VL-102 was also selected and validated as potent activator of cGMP/CREB. VL-102 induced hyperalgesia in mice, mimicking a validated animal model responsive to clinical migraine drugs. In this model, cGMP inhibition by acute treatment with RG2-12 reversed both acute and chronic hyperalgesia, and also reversed the actions of VL-102. To closely mimic the patterns seen in epileptic high-spiking human neocortex, we reproduced a rapid sustained MAPK/CREB-mediated gene transcription using repeated depolarization of SH-SY5Y cells to select potent cGMP/CREB inhibitors for intervention in epileptogenesis. Together, our data indicate that cGMP/CREB signaling is a promising therapeutic target for the chronic or acute treatment of multiple systems associated with neurological insults.

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Poster

053. Neurodegeneration Drug Discovery: Other

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.04/H1

Topic: C.19. Drug Discovery and Development

Support: BMBF grant #13EX0905 (Germany)

Title: Identification and characterization of a novel and highly potent agonist of the human Smoothened receptor

Authors: *J. A. VAN BERGEIJK[1], L. A. SMYTH[2], S. RENNER[1], A. HEUTLING[2], M. SCHMIDT[1], A. H. MEYER[1];
[1]Biol. Dept., [2]Medicinal Chem. Dept., Abbvie Deutschland GmbH & Co. KG, Ludwigshafen, Germany

Abstract: The Hedgehog pathway plays a fundamental role in embryogenesis and becomes silent during adulthood. In adolescence distinct activation can be observed mediating proliferation, migration and differentiation of stem as well as precursor cells. In CNS, pathway stimulation induces adult neurogenesis in the subgranular zone of the dentate gyrus, subventricular zones as well as in non-classical neurogenic regions like the cerebral cortex. Neuroregenerative effects have been reported for the endogenous ligand of Patched, Shh, and low molecular weight agonists of the Smoothened receptor (Smo) downstream of Patched. Disease modification has been demonstrated *in vivo*, most notably in neurodegenerative animal models of Parkinson's disease and trisomy. So far, all potent *and* efficacious Smo agonists are derivatives of chlorobenzothiophene, a core structure initially identified by Frank-Kamenetsky *et al.* in 2002 (SAG1.1 - SAG1.7, CUR201807). Here, we report identification of a novel highly potent and efficacious Smo agonist which we derived from Purmorphamine. Compound Y is chemically distinct from chlorobenzothiophenes and allows a non-biased approach for pathway analysis. In contrast to Purmorphamine, the new activator has a high affinity for hSmo ($K_i^{Cmpd\ Y}$: 60 nM vs. $K_i^{Purm.}$: 2690 nM). Regarding osteoblast differentiation of murine mesenchymal C3H10T1/2, it is more efficacious and about 20 fold more potent ($EC_{50}^{Cmpd\ Y}$: 19 nM). Compound Y induces Gli dependent pathway activation in murine NIH3T3 Gli-*bla* (EC_{50}: 12 nM) as well as in a human mesenchymal cell line (EC_{50}: 6 nM). It selectively activates the hedgehog pathway and does not cross-react with Frizzled receptors modulating canonical Wnt / β-catenin signaling. Stimulation of primary rat granule cerebellar precursor cell proliferation *in vitro* indicates that similar to SAG, compound Y may induce neurogenesis *in vivo*.

Disclosures: **J.A. Van Bergeijk:** A. Employment/Salary (full or part-time):; AbbVie 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.; German Ministry for Education and Research (BMBF). **L.A. Smyth:** None. **S. Renner:** None. **A. Heutling:** None. **M. Schmidt:** None. **A.H. Meyer:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

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Topic: C.19. Drug Discovery and Development

Support: VA merit review grant

Title: 7,8-Dihydroxyflavone protects mitochondrial injury by regulating energy metabolism in an animal model of multiple sclerosis

Authors: *T. K. MAKAR[1,2], V. NIMMAGADDA[1,2], P. GUDA[1], S. JUDGE[1,2], D. TRISLER[1,2], C. BEVER[1,2];
[1]Neurol., Univ. of Maryland Baltimore, Baltimore, MD; [2]Multiple sclerosis center of excellence, VA Maryland Hlth. care system, Baltimore, MD

Abstract: Multiple sclerosis (MS) is an autoimmune neuroinflammatory, demyelinating and neurodegenerative disease influenced by genetics and environmental factors. Post-mortem MS studies have reported mitochondrial abnormalities within neurons. The pathophysiological involvement of mitochondrial injury and energy metabolism in MS and EAE (Experimental allergic encephalomyelitis), an animal model is still not clear.7,8-Dihydroxyflavone (DHF), a TrkB agonist, is a neuro protective agent used for different animal models of neurodegenerative diseases. In addition, the capacity of DHF to inhibit mitochondrial injury and regulate mitochondrial energy homeostasis in MS/EAE is unknown. We first explored the mechanism of mitochondrial injury and perturbation of energy homeostasis in C57 BL/6 EAE mice. Furthermore, we explored the effects of DHF on mitochondrial protection and balancing energy metabolism in EAE. Treatment with DHF (5mg/kg, I.P, daily for 30 days) in EAE mice inhibited clinical severity and inflammation. Treatment with DHF increased TrkB activation after EAE. Improvement in EAE, suggests a pivotal role of TrkB signaling in anti-inflammatory performance after EAE. A potential action of DHF on mitochondrial injury and neuronal energy homeostasis was balanced by the normalization in levels of PGC-1α, Nrf1, TFAM, Drp-1, Mfn2, PINK 1 and SIRT1 in EAE mice. These results suggest a potential mechanism by which DHF counteracts EAE pathology via activation of the TrkB receptor and engaging the interplay between cell energy balance and mitochondrial protection. Since metabolic dysfunction is an important factor for the development of neurodegeneration in MS/EAE, these results set a precedent for the therapeutic use of DHF in EAE and perhaps MS.

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Poster

053. Neurodegeneration Drug Discovery: Other

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Program#/Poster#: 53.06/H3

Topic: C.19. Drug Discovery and Development

Support: NIH/NINDS intramural Funds

Title: Computer modeling for binding prediction and characterization of cdk5-p5 association

Authors: A. CARDONE[1,2], A. HASSAN[3], M. BRADY[1], R. SRIRAM[1], *H. C. PANT[4]; [1]Information technology Laboratory, Software and Systems Division, NIST, Gaithersburg, Maryland 20899, NIST, Gaithersburg, MD; [2]Inst. for Advanced Computer Studies, Univ. of Maryland, College Park, MD; [3]c Div. of Computat. Bioscience, Ctr. for Mol. Modeling, CIT, Natl. Inst. of H, [4]LNC NINDS, NIH, Bethesda, MD

Abstract: The cyclin-dependent kinase Cdk5 plays a fundamental role in mature nervous system development and function. Cdk5 physiological activity is regulated by its activator, p35. Under neuronal insults such as A-beta toxicity as well as Ca2+-induced oxidative stress, p35 is proteolyzed into the p25 and 10 kDa. P25 binds with Cdk5, deregulates and hyperactivates its activity and induces plaques and neurofibrillary tangles, hallmarks of AD pathology. Past research efforts have directly targeted the phosphorylation mechanism, for instance by preventing ATP-binding by the kinase inhibitors. However, this might cause toxic effects due to their ability to bind all the kinases. Our *in situ* experimental conditions showed that truncated fragments from p35 effectively inhibit the Cdk5-p25 activity. The smallest fragment derived from p25 is p5, a 24 amino acid peptide appears to be an ideal therapeutic candidate since it selectivity inhibited Cdk5-p25 (deregulated) compared to the physiological Cdk5-p35 activity. Thus does not induce toxicity. Furthermore, TFP5, a p5-modified peptide, can cross the blood brain barrier and reduced the Alzheimer disease phenotypes in model mice. Recent developments in computer modeling of complex formation are used here to study cdk5-p5 association. The method is designed to detect and characterize non-specific binding and sparsely populated modes, and accounts for both conformational selection and mutually induced fit. A high-affinity, high-population Cdk5-p5 binding mode could be identified as inhibitory as it competes with p25 for binding. Its main characteristics, including electrostatics, hydrophobic, and hydrogen bond patterns, are reproduced in several independent simulations, providing statistical confidence in the data. The pharmacophore was characterized, revealing a set of interactions that can be reproduced by small, drug-like compounds. A subset of amino acids at the cdk5/p5 interface was also identified, which provide valuable information for targeted mutagenesis studies in order to map the binding site experimentally.

Disclosures: A. Cardone: None. **A. Hassan:** None. **M. Brady:** None. **R. Sriram:** None. **H.C. Pant:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

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Program#/Poster#: 53.07/H4

Topic: C.19. Drug Discovery and Development

Support: NIH / NCCAM grant 1PO1AT004511

James J. Peters Veteran's Affair

Title: Targeting inflammation and synaptic plasticity for the treatment of stress disorder and depression

Authors: *J. WANG[1,2], G. HORDES[3], S. GOLDEN[3], W. BI[3], W. ZHAO[3], L. HO[3], S. RUSSO[3], G. M. PASINETTI[3,2];
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Abstract: Depression is a multifaceted mood disorder linked to various mechanisms that are poorly understood. There is a long-standing need for improved anti-depressant treatment. We found that dietary supplementation with a bioactive dietary polyphenol preparation (BDPP) significantly promotes resilience to depression/anxiety phenotypes in a repeated social defeat stress (RSDS) model of depression. Bioavailability studies identified a panel of phytochemicals in the brain and plasma following oral administration of BDPP, including polyphenol metabolites through xenobiotic metabolism and colonic microbiome-derived phenolic acids. Based on our previous observation of stress-mediated long-term disruptions in nucleus accumbens (NAc) medium spiny neuron (MSN) synaptic plasticity and induction of IL-6 in the periphery, which are key contributory factors to depression/anxiety phenotypes, we screened these phytochemicals for their impacts on modulating IL-6 expression in peripheral blood mononuclear cells and/or neuronal synaptic plasticity in MSN-enriched primary neuron cultures. We selected one phytochemical that is potent in attenuating IL-6 expression and one that is potent in promoting synaptic plasticity and conducted *in vitro* discovery stage toxicity and drug-like properties studies and *in vivo* toxicology and efficacy studies. We found that combination therapy with the two phytochemicals targeting periphery proinflammation and CNS synaptic plasticity mechanisms of depression significantly attenuates RSDS-induced depression phenotypes, including improving social interaction and reducing anhedonia, compared to vehicle treated control mice, and these improvements coincide with reduced peripheral IL-6 and increased synaptic plasticity in the NAc. Our studies provide the first experimental evidence that targeting multiple pathologic mechanisms underlying depression may be a viable novel treatment strategy. Based on the safety and drug-like properties of these two phytochemicals, our study can immediately translate into human clinical studies for the treatment of stress disorders and depression.

Disclosures: **J. Wang:** None. **G. Hordes:** None. **S. Golden:** None. **W. Bi:** None. **W. zhao:** None. **L. Ho:** None. **S. Russo:** None. **G.M. Pasinetti:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

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Support: Leading talents in entrepreneurship of Guangzhou Municipal Government（Startup project LCY201306）

Leading talent project in science and technology of Guangzhou Development (Project No: 2013 L-p090)

Introduction of Innovative R&D team Program of Guangdong Province (NO.2013Y104)

Title: A synthetic steroid 5α-androst-3β,5,6β-triol functions as a novel neuroprotectant against ischemic stroke via multiple mechanisms

Authors: ***S. LIN**[1], Y. HUANG[2], Y. ZHOU[2], J. CHEN[2], T. LENG[3], H. HU[4], M. YAN[2], L. TANG[2], Y. LI[2], P. QIU[2], W. YIN[2], J. ZHANG[4], Z. XIONG[3], D. DUAN[5], J. LIN[6], H. SHI[1], Y. WANG[1], G. YAN[2];
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Abstract: Our previous studies reported that, a synthetic steroid 5α-androst-3β,5,6β-triol (codename YC-6) is neuroprotective against hypoxia/reoxygenation induced neuronal injury *in vitro*. In the current study, we examined the neuroprotective effect of YC-6 *in vivo*. The effect of YC-6 was tested in a transient middle cerebral artery occlusion (MCAO) model of ischemia in rats. We found that administration of Triol 15 minutes prior to 2h MCAO dose-dependently reduced the infarction volume quantified by 2,3,5-triphenyltetrazolium chloride (TTC) staining and improved the neurological score as well as behavior outcomes. Remarkably, the therapeutic time window for YC-6 could be extended to 5h. Furthermore, we examined the mechanisms underlying the neuroprotective effect of YC-6. Our results showed that 1) YC-6 protected against

glutamate-induced excitotoxicity in both primary cultured cortical neurons and cerebella granule neurons in SD rats through inhibition of glutamate-mediated cytoplasmic calcium overload and acidosis. 2) YC-6 attenuated the acid sensing ion channel (ASIC) currents in cortical neurons. 3) YC-6 suppressed the release of proinflammatory factors and suppressed the inflammatory pathways via inhibition of microglia activation in primary cultured microglia. In summary, these results suggest that YC-6 is a powerful and effective neuroprotectant that can simultaneously and synergistically target at multiple mechanisms of ischemic stroke-induced injuries, including glutamate-mediated [Ca2+]i overload and acidosis, ASICs, and inflammatory pathways. Therefore, YC-6 may be a novel neuroprotectant and a promising drug candidate for the treatment of ischemic stroke.

Disclosures: **S. Lin:** None. **Y. huang:** None. **Y. zhou:** None. **J. chen:** None. **T. leng:** None. **H. hu:** None. **M. yan:** None. **L. tang:** None. **Y. li:** None. **P. qiu:** None. **W. yin:** None. **J. zhang:** None. **Z. xiong:** None. **D. duan:** None. **J. lin:** None. **H. shi:** None. **Y. wang:** None. **G. yan:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

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PMU-FFF grant E-10/11/056-COU

FWF Special Research Program (SFB) F44-13

Title: Structural and functional rejuvenation of the aged and of the neurodegenerative brain by an approved anti-asthmatic drug

Authors: J. MARSCHALLINGER[1], B. KLEIN[1], I. SCHÄFFNER[2], R. GELFERT[1], S. ILLES[1], L. GRASSNER[1], M. JANSSEN[1], P. ROTHENEICHNER[1], C. SCHMUCKERMAIR[3], R.

CORAS[4], M. BOCCAZZI[5], M. CHISHTY[6], F. LAGLER[1], M. RENIC[7], F. RIVERA[1], H.-C. BAUER[1], N. SINGEWALD[3], I. BLÜMCKE[4], U. BOGDAHN[8], S. COUILLARD-DESPRES[1], D. C. LIE[2], E. ROCKENSTEIN[9], E. MASLIAH[9], M. P. ABBRACCHIO[5], *L. J. AIGNER[1]; [1]Paracelsus Med. Univ., Salzburg, Austria; [2]Res. Group Adult Neurogenesis and Neural Stem Cells, Inst. of Developmental Genet., Helmholtz Zentrum München, German Res. Ctr. for Envrn. Health,, Munich, Germany; [3]Leopold-Franzens-University of Innsbruck, Innsbruck, Austria; [4]Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany; [5]Univ. of Milan, Milan, Italy; [6]Pharmidex, London, United Kingdom; [7]Univ. of Zagreb Sch. of Med., Zagreb, Croatia; [8]Univ. Hosp. Regensburg, Regensburg, Germany; [9]UCSD, San Diego, CA

Abstract: As human life expectancy has improved rapidly in industrialized societies, counteracting age-related cognitive impairment presents an increasing challenge. Histopathological hallmarks correlating with age-related cognitive declines are, among others, neuroinflammation, low levels of neurogenesis, blood-brain barrier disintegrity, and altered neuronal activity. While targeting such age-related processes, for example by exposure to a young systemic environment, i.e. young blood through heterochronic parabiosis, indeed leads to structural and functional rejuvenation of the aged brain, clinical translation of the brain rejuvenation concept is still awaiting evidence. Here, we identified leukotriene receptor signalling as a safe and druggable pathway with a combined mode of action to reduce neuroinflammation and to enhance neurogenesis, specifically in the aged brain. Treatment with montelukast, an already marketed anti-asthmatic drug acting on leukotriene receptors, successfully rejuvenated the aged brain and restored cognitive function in aged animals. Moreoever, similar regenerative and cognition-stimulating effects were obtained by treatment of a genetic Parkinsons disease animal model with montelukast. This paves the way to future clinical translation for the treatment of neurodegenerative diseases and dementias.

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Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.10/H7

Topic: C.19. Drug Discovery and Development

Support: MS Society Fast Forward Grant

Title: NDC-1308, a small molecule with remyelinating activity for treatment of secondary progressive multiple sclerosis patients

Authors: *S. H. NYE, J. G. YARGER;
Discovery, ENDECE Neural, LLC, Mequon, WI

Abstract: Background: Current therapeutics for MS patient's impact the immune mechanisms of the disease, rather than the elusive remyelination activity needed to repair the damaged myelin sheath. NDC-1308 is known to activate intracellular pathways for oligodendrocyte progenitor cell (OPC) differentiation. NDC-1308 induces mouse OPCs to differentiate into mature, myelinating oligodendrocytes *in vitro* and significantly increases remyelination of axons *in vivo*. These studies extend the *in vivo* remyelination results while addressing the safety and exposure of NDC-1308. Objectives: A main goal was to correlate the ability of NDC-1308 to repair the damaged myelin sheath, in the cuprizone mouse model of demyelination, with a functional improvement. Intended outcomes also included an initial safety assessment for NDC-1308 and determining the dosing parameters for non-clinical IND-enabling studies. Methods: Male mice were treated for 12-weeks with cuprizone and rapamycin to cause demyelination of white and gray matter regions of the brain. The demyelinated mice were administered NDC-1308 (68 mg/Kg, i.p., q.d.) for up to 6 weeks. Blood was collected at termination for clinical chemistry analysis, along with reproductive tracts for pathology, and brain regions for assessing the level of NDC-1308 remyelination. The OPC population in different brain regions was evaluated by immunohistochemistry using PDGFRα antibodies. The degree of mutagenicity and genotoxicity of NDC-1308 was measured by a bacterial reverse mutation assay and a mammalian cell micronucleus screening assay, respectively. Results: NDC-1308 rapidly crosses the blood brain barrier and is absorbed into CNS tissues at levels exceeding the EC50 required for OPC differentiation *in vitro*. NDC-1308 is eliminated from the CNS and periphery after 24 hours. Following chronic treatment of demyelinated mice with NDC-1308, remyelination was significantly increased 18% ($P<0.01$) in cortical regions and 44% ($P<0.0001$) in hippocampal regions. A significant increase in grip strength was measured in the animals treated with NDC-1308. Observed animal behavior and clinical chemistries were normal and the OPC population remained intact. NDC-1308 was found to be non-mutagenic and non-genotoxic. Conclusions: These results suggest NDC-1308 can be delivered to the CNS tissues in amounts sufficient for inducing OPCs to differentiate into mature, myelinating oligodendroctyes that can then repair the myelin sheath. NDC-1308 appears to be a potentially safe and effective therapeutic for treating SPMS patients. Non-clinical IND-enabling studies and a first-in-human Phase 1 study are planned.

Disclosures: **S.H. Nye:** A. Employment/Salary (full or part-time):; Full time, ENDECE Neural, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ENDECE Neural, LLC. **J.G. Yarger:** A. Employment/Salary (full or part-time):; ENDECE Neural, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ENDECE Neural, LLC.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

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Program#/Poster#: 53.11/H8

Topic: C.19. Drug Discovery and Development

Support: Stanley Foundation

Swedish Medical Research Council (MFR)

The Swedish Brain Foundation

Title: ISP - An *in vivo* based systems pharmacology platform for phenotypic characterization of CNS treatments, translational modelling and drug discovery

Authors: ***P. SVENSSON**, S. WATERS, C. SONESSON, E. LJUNG, B. SVANBERG, N. WATERS;
Integrative Res. Labs. (IRL), Gothenburg, Sweden

Abstract: The CNS is a complex biological system, and as such, inherently prone to degeneracy of functions. This promotes robustness and flexibility of any complex biological system. However, this property limits the efficiency of highly reductionist approaches to understand physiological and pharmacological mechanisms in healthy or in disease states. A manifestation of this problem is that phenotypic screening has proven more successful than target based screening in generating first-in-class drugs within the CNS area (1). In order to overcome this limitation we developed a standardized process, called Integrative Screening Process (ISP), for *in vivo* systems-response profiling, using a comprehensive array of biomarkers, as a way to obtain a sensitive fingerprint of the functional, network level effects of CNS-acting compounds. The response profiles provide a basis for comparison and classification of CNS compounds and for predicting potential clinical properties of new agents, beyond what can be achieved using single-endpoint *in vivo* or *in vitro* methodologies. ISP can be described as an established, efficient and

validated *in vivo* based systems pharmacology platform for phenotypic characterization of CNS treatments and translational modelling. It has been systematically implemented and integrated throughout our drug discovery process. ISP has been developed and utilized for more than a decade, resulting in a database covering *in vivo* systems response profiles of approximately 900 in-house compounds and more than 300 reference compounds with documented CNS effects in humans. During this decade ISP has generated 8 candidate drugs, of which three are in late stage preclinical development, two are in in phase I, and one is in late stage development for Huntington's Disease. Key features of ISP such as standardization/comparability, translational ability and predictivity, ability to capture direct and indirect effects as well as state dependent effect will be discussed. Experimental and computational methodology, QC aspects, utility in classification, QAAR and QSAR, and data analytical strategies for translational modelling will be described and discussed. References: (1)Swinney DC, Anthony J. How were new medicines discovered? Nat Rev Drug Discov. 2011 Jun 24;10(7):507-19

Disclosures: **P. Svensson:** A. Employment/Salary (full or part-time):; Integrative Research Laboratories (IRL). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories (IRL). **S. Waters:** A. Employment/Salary (full or part-time):; Integrative Research Laboratories (IRL). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories (IRL). **C. Sonesson:** A. Employment/Salary (full or part-time):; Integrative Research Laboratories (IRL). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories (IRL). **E. Ljung:** A. Employment/Salary (full or part-time):; Integrative Research Laboratories (IRL). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories (IRL). **B. Svanberg:** A. Employment/Salary (full or part-time):; Integrative Research Laboratories (IRL). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories (IRL). **N. Waters:** A. Employment/Salary (full or part-time):; Integrative Research Laboratories (IRL). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrative Research Laboratories (IRL).

Poster

053. Neurodegeneration Drug Discovery: Other

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Title: Prediction of glutamatergic neurotoxicity of drugs and pollutants by biosimulation: cholinesterase inhibitors (AChEI) and metallic nanoparticles (MNPs)

Authors: R. GREGET[1], L. BARBIER[2], S. DADAK[3], F. LALOUE[1], J.-M. C. BOUTEILLER[1,4,5], L. FAGNI[3], F. DORANDEU[2], *S. BISCHOFF[1,5], M. BAUDRY[1,5,6], S. MOUSSAOUI[1];
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Abstract: Anticipating safety issues early on in the drug discovery process would greatly improve its success rate. Similarly, identifying neurotoxic risks of environmental factors would help to better understand their impact on human and animal health. To specifically address these two aspects, we developed two new simulators validated by *in vitro* and *in vivo* experiments. A first simulator was created in the framework of a Defense program (*) aiming at studying neuronal hyperexcitability induced by irreversible organophosphate inhibitors of acetylcholinesterase (AChEIs), such as Paraoxon (POX). This new tool integrates morphological and electrochemical properties of hippocampal CA1 neurons, interneurons (OLM and Basket cells), glutamatergic afferents from entorhinal cortex and hippocampal CA3 neurons, as well as cholinergic afferents from the septum. Synapses comprise many models of receptors, channels, second messenger systems as well as acetylcholinesterase (AChE) and muscarinic M1R coupled to the potassium channel Kv7. This simulator reproduced the effects of POX on hyperexcitability and occurrence of epileptiform patterns. It allowed developing strategies to counteract POX neurotoxic effects, in particular by identifying drug combinations, which could reduce side effects or serve as antidotes to protect against chemical weapons. A second simulator developed in the framework of the EC program (**) integrates mitochondria and the p53 and NFkB pathways to address the biology and toxicity of metallic ZnO and SiO_2 nanoparticles (MNPs). These different models, interconnected with each other and with a pharmacokinetic model enable studying the dose-, size- and time-dependent effects of MNPs on diverse biological reactions, targets and pathways, such as dehydrogenase flux, electron transport system fluxes (Complex I, III and IV), substrate transport fluxes (ATP, ADP, AMP, and inorganic phosphate), adenine nucleotide translocase flux (ANT flux), superoxide production and transport (IMAC), superoxide

dismutase (SOD) and catalase (CAT) activity, glutathione (GSH), P53 activation, and NFκB activation, as regulated by three IκB isoforms (IκBα, IκBβ, and IκBε). These new simulators can be used alone or in combination with our existing physiological and pathological multilevel and translational simulators. They are available to perform specific studies for industrial, government, foundation and academic research partners.

Disclosures: R. Greget: None. **L. Barbier:** None. **S. Dadak:** None. **F. Laloue:** None. **J.C. Bouteiller:** None. **L. Fagni:** None. **F. Dorandeu:** None. **S. Bischoff:** None. **M. Baudry:** None. **S. Moussaoui:** Other; New affiliation, Firalis, Huningue, France.

Poster

053. Neurodegeneration Drug Discovery: Other

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Topic: C.19. Drug Discovery and Development

Support: NIH grant NS R01084817

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Title: Combined Ca^{2+} channel/NMDAR antagonism reduces excessive voltage-gated Ca^{2+} influx in pyramidal neurons from the medial prefrontal cortex of HIV-1 transgenic rats

Authors: *C. KHODR[1], S. DAVE[1], L. CHEN[1], C. ZHANG[1], L. AL-HARTHI[2], X.-T. HU[1]; [1]Dept. of Pharmacol., [2]Dept. of Immunol. and Microbiology, Rush Univ. Med. Ctr., Chicago, IL

Abstract: HIV-1 infection is associated with impaired neurocognitive function that is regulated by the medial prefrontal cortex (mPFC) and other HIV-vulnerable brain regions. Ca^{2+} dysregulation, especially excessive Ca^{2+} influx which increases cytosolic Ca^{2+} levels, is a significant risk factor for neurotoxicity associated with chronic exposure to HIV. Neuronal Ca^{2+} influx is mainly regulated by the NMDA receptor (NMDAR) and voltage-gated Ca^{2+} channels (including the L-type Ca^{2+} channel, L-channel). Use of NMDAR antagonist or L-channel blocker alone to treat HIV dementia complex has either failed or shown limited improvement in clinical trials, suggesting that novel therapeutic strategies (including combined NMDAR/L-channel antagonism in earlier disease states) may be needed to reduce Ca^{2+} related toxicity in neuroAIDS. Our recent studies showed that *in vivo* exposure to HIV-1 proteins induces hyper-excitation of mPFC pyramidal neurons due in part to increased, NMDAR-independent Ca^{2+} influx, using

HIV-1 transgenic (Tg) rats that express 7 of the 9 HIV-1 genes. L-channels are involved in this hyper-excitation because enhanced Ca^{2+} influx was abolished by acute L-channel blockade *in vitro.* Here we evaluated whether combined chronic blockade of over-active L-channels and NMDARs *in vivo* would reduce excessive Ca^{2+} influx due to HIV exposure. Five to six week old male non-Tg and HIV-1 Tg rats received daily s.c. injections of saline (SAL) or diltiazem (15 mg/kg, a selective L-channel blocker) plus memantine (10 mg/kg, a selective NMDAR blocker) for 2 weeks. Whole-cell current-clamp recordings were performed on mPFC pyramidal neurons in brain slices to assess voltage-gated Ca^{2+} influx reflected by evoked Ca^{2+} plateau potentials. During experiments, all neurons were studied under blockade of glutamate and $GABA_A$ receptor mediated synaptic activities, as well as voltage-gated Na^+ and K^+ channels. We found that neurons from SAL-pre-treated HIV-1 Tg rats displayed significantly longer Ca^{2+} potentials (2752±341 ms) measured at the half spike amplitude compared to those from SAL-pretreated non-Tg rats (1619±243 ms, $p \leq 0.01$). Combined chronic blockade of L-channel/NMDAR significantly reduced this increased Ca^{2+} influx in neurons from HIV-1 Tg rats (1866±225 ms, $p \leq 0.05$). No significant difference was found in the half-peak duration of Ca^{2+} potentials between neurons from HIV-1 Tg rats pre-treated with combined blockers and those from non-Tg rats pre-treated with SAL (1866±225 *vs.* 2268±339 ms, $p > 0.05$). Collectively, these findings demonstrate promising effects of combined L-channel/NMDAR antagonism on reducing excessive neuronal Ca^{2+} influx induced by HIV.

Disclosures: C. Khodr: None. **S. Dave:** None. **L. Chen:** None. **C. Zhang:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

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Topic: C.19. Drug Discovery and Development

Support: NIH grant NS085689

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Title: Human stem cell-based high-throughput screening platform for neurodegenerative diseases

Authors: *Z.-W. DU, S.-C. ZHANG;
Waisman Cntr, Univ. Wisconsin, Madison, WI

Abstract: Drug screening for neurodegenerative disease often employ fibroblasts or lymphocytes immortalized from patients and ectopic reporter transgene, leading to high failure rates in clinical trials. The selective vulnerability of subtype-specific neuron is a defining feature of neurodegenerative disease. Therefore, subtype-specific neurons are more relevant target cells for drug screening. In this study, we established disease iPSC reporter lines by CRISPR technology to target only one copy of a sensitive reporter (Nanoluc) into the endogenous disease related gene locus, such as SMN2 gene for spinal muscular atrophy and NEFL gene for amyotrophic lateral sclerosis. Combined with our recently developed method to produce large-scale and pure subtype-specific neurons from iPSCs, we have established a neuron based high-throughput screening (HTS) platform. The HTS platform is optimized in a 384-well format, validated with previously identified compounds and applied for primary screening with NIH clinical collection library. These results suggest that human stem cells can be readily engineered and formatted for phenotypic and mechanistic HTS screening for neurodegenerative diseases

Disclosures: **Z. Du:** A. Employment/Salary (full or part-time):; BRAINXELL. **S. Zhang:** A. Employment/Salary (full or part-time):; BRAINXELL.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.15/H12

Topic: C.19. Drug Discovery and Development

Title: Extracellular zinc changes the kinetics of streptokinase in thrombolysis *in vitro*

Authors: *Z. WANG, Y. LI;
Biomed. Sci., Ohio Univ., Athens, OH

Abstract: Thrombotic ischemic stroke occurs when local blood clot obstructs an artery to brain, causing neurons lose function and die. It is one of the leading causes of mortality and a major cause of disability. The effect of thrombolysis by injecting intravenous thrombolytic agents is critical for reducing stroke damages. Streptokinase (SK) is a widely used thrombolytic agent in the treatment of thromboembolism in the blood vessels. High unit of SK was used in thrombolytic therapies of myocardial ischemia and could improve tissue reperfusion. It is a potent plasminogen activator. However, safety concerns of usage of high unit of SK are still noticed. In our present study, we performed on the effect of SK and how zinc affect SK-induced thrombolysis *in vitro* studies, and proposed a strategy to improve SK effectiveness in thrombolysis. The mice whole blood was used to produce blood clot *in vitro* by incubated with

calcium at 37C for 3h. SK was used for inducing thrombolysis. Zinc and its chelator, CaEDTA, were applied with SK respectively. Spectorphotometer was used to measure thrombolysis effect at 580 nm wavelength. Results showed that SK had dose dependence effect and exhibited thrombolysis effect after 60 minutes. Zinc inhibited thrombolysis effect of SK. Zinc chelator, CaEDTA, significantly increased the effect of SK-induced thrombolysis. We concluded that zinc inhibit SK effect in blood clot lysing *in vitro*. Chelation of zinc improves SK effectiveness in thrombolysis.

Disclosures: Z. Wang: None. **Y. Li:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

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NIH Grant AG031311

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ADDF award 261108.01

Title: Addressing innovation in CNS drug discovery: preclinical proof of concept for parallel campaigns that employ either functional or single molecular target approaches for delivery of novel candidates that attenuate synaptic and cognitive dysfunction

Authors: *D. WATTERSON[1], O. ARANCIO[2], S. M. ROY[1], L. VAN ELDIK[3];
[1]Dept. of Pharmacol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [2]Columbia Univ., New York, NY; [3]Univ. of Kentucky, Lexington, KY

Abstract: Diverse CNS disorders display common pathophysiology themes. Ongoing efforts to develop disease-modifying therapeutics reflect the increasing appreciation of these core mechanisms. Major challenges include identification of disease progression time windows amenable to intervention, the linkage of disease related phenotypes to druggable molecular targets, and the need for clinical landmarks. Retrospective analyses of new molecular entity drug

approvals reveal the differing impact of phenotypic vs single molecular target drug discovery approaches, dependent on the state of knowledge about druggable disease progression mechanisms. The less biased phenotypic approach allows probing for interventions in the absence of established mechanisms in areas of critical unmet medical need, whereas single molecular target approaches provide exceptional efficiencies in the context of established mechanisms. As part of a multi-site collaboratorium focused on innate immunity and synaptic dysfunction as a common pathophysiology progression theme, we explored the potential for novel small molecule drug candidate discovery using these two distinct approaches and a common molecular scaffold or fragment. Specifically, the functional approach focused on early stage overproduction of proinflammatory cytokines causally linked to synaptic dysfunction and the single molecular target was brain p38aMAPK, an established regulator of innate immunity in glia and an intracellular neuronal kinase up-regulated in stress responses. Our goal was to start with: 1) an *in vivo* screening approach based on disease-relevant pharmacodynamic end points (functional approach), or 2) a structure-assisted, pharmacoinformatics-driven design (p38aMAPK). An aminoarylpyridazine molecular fragment was the common core. Deliverables from each approach used a common secondary phase, pharmacology driven medicinal chemistry refinement. Novel small molecule deliverables from each approach showed efficacy in diverse preclinical animal models. Both showed *in vivo* attenuation of innate immunity related pathologies and behavioral deficits. Novel candidates are in preclinical or clinical drug development.

Disclosures: **D. Watterson:** None. **O. Arancio:** None. **S.M. Roy:** None. **L. Van Eldik:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.17/H14

Topic: C.19. Drug Discovery and Development

Support: Les Turner ALS Foundation

Foglia Family Foundation

Northwestern Center for Molecular Innovation and Drug Discovery

The Les Turner ALS Foundation/Herbert C. Wenske Professorship

Questcor Pharmaceuticals

Title: Etiology-based drug discovery is a route for new amyotrophic lateral sclerosis (ALS) therapeutics

Authors: *T. J. LUKAS[1], H. ARRAT[2], T. SIDDIQUE[2];
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Abstract: The treatment of neurodegenerative diseases is difficult because of multiple etiologies and the interplay of genetics and environment as precipitating factors. In the case of amyotrophic lateral sclerosis (ALS), we have knowledge of of several genes that cause this fatal disease when mutated. However, drugs to counteract the effect of genetic mutations have not yet been found. One of the causative genes, Cu, Zn-superoxide dismutase (SOD1) is responsible for about 10-15% of the genetically linked autosomal dominant disease. Our rationale was that compounds that reduce expression of the toxic protein would be beneficial to slow onset and/or disease progression. We screened candidate compounds using a cell-based *in vitro* assay for those that reduce mutant SOD1 (G93A) protein levels. This led to the discovery of 2-[3-iodophenyl)methylsulfanyl]-5pyridin-4-yl-1,3,4-oxadiazole, a known protein kinase inhibitor that decreases G93A-SOD1 levels *in vitro* and in the brain and spinal cord of mice *in vivo*. However, this compound has a biphasic dose response curve and a potential toxophore which limits its therapeutic window for chronic disease such as ALS. Therefore, we designed and tested a focused library of analogues for their ability to decrease SOD1 expression *in vitro*. This exercise resulted in the identification of two new lead compounds with improved drug-like characteristics and activity. Currently, optimization of delivery vehicle and route are in progress for preclinical testing in the G93A-SOD1 mouse model of ALS. In the course of the preliminary testing of another agent for efficacy in the G93A-SOD1 mouse we unexpectedly found that ACTH (Acthar gel, Questcor) also decreased the expression of mutant SOD1 in the mouse spinal cord and brain. Acthar also delayed onset of disease and trended toward slowing of disease progression. Additional preclinical testing in a larger animal cohort is underway to determine whether Acthar can significantly improve survival. Thus, two different agents have been found with the potential for treating SOD1 mutant-linked ALS. Development of small molecules and other agents that reduce the expression of etiologically relevant toxic proteins is a strategy that may also be extended to other familial ALS genes for which a toxic gain of function leads to disease.

Disclosures: **T.J. Lukas:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Questcor Pharmaceuticals. **H. Arrat:** None. **T. Siddique:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.19. Drug Discovery and Development

Support: RGC GRF Grant 460712

RGC GRF Grant 461013

RGC GRF Grant 14100714

RGC CRF Grant CUHK1/CRF/13G

RGC/ANR Joint Research Scheme A-CUHK401/14

HMRF Grant 01120626

CUHK Group Research Scheme 3110102

Title: A peptidylic inhibitor approach to suppress polyglutamine disease toxicity

Authors: *E. CHAN, T. ZHANG, H. TSOI, I. PENG, K.-F. LAU, J. NGO;
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Abstract: Polyglutamine (polyQ) diseases are dominantly inherited neurological disorders caused by CAG trinucleotide insertion mutation in the human genome. These diseases are characterized by progressive neuronal cell dysfunction and cell death. Polyglutamine disease toxicity is attributed to the neuronal expression of both RNA and protein that respectively carry elongated runs of CAG and glutamine repeats. We report the identification of a peptide sequence which targets CAG RNA toxicity. Mechanistically, our inhibitor suppresses the nucleolar stress-induced caspase activation triggered by elongated CAG RNA. Our findings raise the possibility of targeting both RNA and protein toxicities of polyQ degeneration.

Disclosures: E. Chan: None. **T. Zhang:** None. **H. Tsoi:** None. **I. Peng:** None. **K. Lau:** None. **J. Ngo:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.19/H16

Topic: C.19. Drug Discovery and Development

Support: Supported by the Altschul Foundation to GMP.

Title: Shared genetic etiology underlying Alzheimer's disease and type 2 diabetes

Authors: K. HAO[1], A. F. DINARZO[1], W. LUO[1], S. LI[1], R. CHEN[1], L. HO[2], *G. M. PASINETTI[3,4];
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Abstract: Epidemiological evidence supports the observation that subjects with type 2 diabetes (T2D) are at higher risk to develop Alzheimer's disease (AD). However, how/if these two conditions are causally linked is unknown. Possible mechanisms include shared genetic risk factors, which we investigated in this study based on recent genome wide association study (GWAS) findings. In order to achieve our goal, we retrieved single nucleotide polymorphisms (SNPs) associated with AD and T2D from large-scale GWAS meta-analysis consortia and tested for overlap among the AD- and T2D-associated SNPs at various p-value thresholds. We then explored the function of the AD-T2D shared GWAS SNPs by leveraging expressional quantitative trait loci, pathways, gene ontology data, and co-expression networks. We found 927 SNPs associated with both AD and T2D with pvalue≤0.01, an overlap significantly larger than random chance (p-value=6.93E-28). Among these, 395 of the shared GWAS SNPs have the same risk allele for AD and T2D, suggesting common pathogenic mechanisms underlying the development of AD and T2D. Most excitingly, we found that 532 of the AD-T2D shared GWAS SNPs had divergent risk alleles in the two diseases, suggesting they play opposite pathogenic roles underlying AD and T2D etiology in the brain and in the peripheral organs. Collectively, our GWAS studies tentatively support the epidemiological observation of the disease incidence correlation between T2D and AD. Moreover, the studies provide the much needed information for the design of future novel therapeutic approaches that might benefit T2D without promoting further deterioration of AD neuropathology and cognitive functions.

Disclosures: K. Hao: None. **A.F. DiNarzo:** None. **W. Luo:** None. **S. Li:** None. **R. Chen:** None. **L. Ho:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LH is among the inventors of a patent using grape seed extract (GSE) in neurodegenerative diseases (Patent number 8747924; Methods for preventing and treating neurodegenerative diseases). **G.M. Pasinetti:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GMP is among the inventors of a patent using grape seed extract (GSE) in neurodegenerative diseases (Patent number 8747924; Methods for preventing and treating neurodegenerative diseases)..

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.20/H17

Topic: C.19. Drug Discovery and Development

Support: NIDA Grant DA9158

NIDA Grant DA3801

Title: Exploration of human cannabinoid 2 receptor binding site using AM1336, a novel covalent ligand

Authors: *S. MALLIPEDDI[1], N. ZVONOK[2], A. MAKRIYANNIS[3];
[2]Pharmaceut. Sci., [1]Northeastern Univ., Boston, MA; [3]Pharmaceut. Sci. and Chem. and Chem. Biol., Northeastern Univ., Northeastern University, MA

Abstract: Cannabinoid 2 receptor (CB2R) belongs to the Class A family of G-protein coupled receptors and is expressed predominantly in the periphery, but to a certain extent in the central nervous system. CB2 receptors, upon activation by endogenous or synthetic ligands, modulate a diverse set of downstream signaling pathways and therefore an attractive drug target for pain management and other neurological and inflammatory disorders. But the lack of a complete experimental characterization of the CB2 receptor structure and its ligand-binding site has hampered rational drug design and development. AM1336 is a novel biarylpyrazole derivative, functionalized with an isothiocyanate group for covalent attachment to cysteine residue(s) at or near the ligand binding site. It acts as a high-affinity, inverse agonist at both cannabinoid receptors, however binds irreversibly only to CB2R. We expressed a full-length human CB2R in insect cells and purified functional receptor using a single-step immunoaffinity chromatography with yields of ~200 ug/L of culture. A modified radioligand binding assay confirmed the efficient labeling of the purified CB2 receptor with AM1336 and a top-down high-resolution mass spectrometry (MS) based proteomics approach identified the modification of cysteines in transmembrane helix 7 (TMH7). This data directly confirms the previously published mutational study results and provides a new MS-based proteomics approach for ligand binding site characterization and guidance for structure-based GPCR ligand design.

Disclosures: S. Mallipeddi: None. **N. Zvonok:** None. **A. Makriyannis:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.21/H18

Topic: C.19. Drug Discovery and Development

Title: Targeting the kynurenine pathway of tryptophan metabolism: a structural biology approach

Authors: *L. PIDUGU[1], B. ROTH[1], C. BEATO[2], S. MALIK[1], R. BADDAM[1], T. L. WANG[1], D. N. PATTERSON[1], K. VARNEY[1], G. COSTANTINO[2], R. SCHWARCZ[3], D. J. WEBER[1], E. A. TOTH[1];
[1]Biochem. and Mol. Biol., CBT, Univ. of Maryland Sch. of Med., Rockville, MD; [2]Dept. di Farmacia, Università degli Studi di Parma, Parco Area delle Scienze 27/A, 43124 Parma, Italy; [3]Maryland Psychiatric Res. Center, Dept. of Psychiatry, Univ. of Maryland Sch. of Medicine,, Baltimore, MD

Abstract: The kynurenine pathway (KP) of tryptophan degradation maintains a delicate balance of neuroactive metabolites such as kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN). 3-HK and QUIN are toxic to neurons, but are also intermediates in a metabolic cascade leading to the ubiquitous co-factor NAD^+. Notably, changes in QUIN levels can affect the levels of other neuroactive metabolites in the pathway, including KYNA (H.-Q. Wu et al., this meeting). KYNA, which is protective against the neurotoxic effects of QUIN, also plays a major role in cognition, and enhanced cerebral KYNA levels cause distinct cognitive impairments. Thus, if the ratio of these KP metabolites is altered beyond a physiological range, the balance of neuroprotection and cognition is shifted, possibly contributing to diseases ranging from Huntington's disease and Alzheimer's disease to schizophrenia and major depressive disorders. The need to maintain homeostasis consequently makes the KP an attractive therapeutic target. As part of our ongoing drug design program, we have so far focused on three KP enzymes: 3-hydroxyanthranilic acid oxygenase (3HAO); quinolinic acid phosphoribosyltransferase (QPRT); and kynurenine aminotransferase II (KAT II). We present here an overview of our recent efforts to characterize these enzymes and relevant ligand complexes thereof by X-ray crystallography and/or NMR. With regard to 3HAO, we report the first structural evidence, by NMR, that the inhibitor 2-amino-6-methylnicotinic acid 1-oxide (UPAR-12) interacts with the human enzyme. TROSY-HSQC of 3HAO indicated a substantial conformational change on binding to this compound. Further, our NMR titrations of 3HAO with Fe^{2+}, Zn^{2+} and EDTA revealed that the bound metal can be controlled, likely due to the presence of a large open cavity that facilitates metal exchange. Comparison of crystal structures of QPRT, i. e. native, in complex with its inhibitor phthalic acid and, most recently, with its product

nicotinic acid mononucleotide, helped us identify the key residues that are involved in structural transitions necessary for the activity of this enzyme. Finally, we describe the crystal structure of human KAT II at a higher resolution (1.69Å) than previously reported. The above observations will aid in structure-based optimization of inhibitor efficacy and thereby in the development of new potential therapeutics.

Disclosures: **L. Pidugu:** None. **B. Roth:** None. **C. Beato:** None. **S. Malik:** None. **R. Baddam:** None. **T.L. Wang:** None. **D.N. Patterson:** None. **K. Varney:** None. **G. Costantino:** None. **R. Schwarcz:** None. **D.J. Weber:** None. **E.A. Toth:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.22/H19

Topic: C.03. Parkinson's Disease

Support: NIH R15 NS048508-02

NSF CCLI 0310627

NSF MRI 0115919

Parkinson's Disease Foundation

Title: Alpha-synuclein familial mutant analysis in yeast models

Authors: *C. ALVARADO[1], M. TEMBO[2], N. KUKULKA[2], M. MUNOZ[2], S. K. DEBBURMAN[2];
[2]Neurosci. Program, [1]Lake Forest Col., Lake Forest, IL

Abstract: Parkinson's disease (PD) is associated with the aggregation and misfolding of alpha-synuclein in midbrain dopaminergic neurons. The gene for alpha-synuclein has six known mutations that directly cause familial forms of PD. The pathological determinants of three mutants (A30P, E46K, and A53T) have been extensively studied and characterized in diverse model systems that indicate that each mutant affects cellular toxicity in distinctive ways. How these three amino acids influence each other's properties is unclear. Unlike these three mutants, the three more recently discovered familial mutants (H50Q, G51D, and A53E) are not extensively studied. First, we created combination mutants of A30P, E46K, and A53T and characterized them in budding and fission yeast models and found support for the dominance of

the A30P mutant over E46K and A53T mutants in both models, shedding new light on the dominance of A30P on alpha-synuclein's conformation. Secondly, we expressed H50Q, G51D, and A53E mutants in both yeasts models and hypothesized that each would generate toxicity by altering membrane-association, and aggregation properties of alpha-synuclein, but each would do so in distinctive ways. We found that the H50Q and A53E mutants were toxic to yeast, and bound membranes and aggregated within yeast, while G51D was cytoplasmically diffuse and non-toxic. Finally, we are currently characterizing combination mutants of the newer mutants in both yeasts. This work adds insight into the pathogenicity of different familial PD mutants of alpha-synuclein.

Disclosures: C. Alvarado: None. **M. Tembo:** None. **N. Kukulka:** None. **M. Munoz:** None. **S.K. DebBurman:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.23/H20

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01 NS083845

Title: Tetramer-abolishing alpha-synuclein variants cause neurotoxicity and inclusion formation: clearance mechanisms and relevance for Parkinson's disease

Authors: *V. VON SAUCKEN, A. J. NEWMAN, T. BARTELS, U. DETTMER, D. SELKOE; Ann Romney Ctr. for Neurologic Dis., Brigham and Women's Hosp. and Harvard Med. S, Boston, MA

Abstract: There is disagreement on what the major physiological structure of alpha-synuclein (aS) is in healthy, intact cells. Our work suggests that aS exists principally as a helically folded tetramer of ~60 kDa and related multimers. We hypothesize that if native multimers are triggered by various genetic or environmental factors to destabilize, unfolded monomers will accumulate in neurons, resulting in toxic aggregates characteristic of Parkinson's disease. To assess the determinants of stability of aS multimers in intact cells, we introduced strategic point mutations in the canonical aS repeat motifs (KTKEGV). We essentially abolished physiological aS multimers by certain (but not all) in-register repeat substitutions, as assessed by the intact-cell methods of chemical crosslinking and YFP complementation. We have evidence of greater neurotoxicity from those aS variants that shift equilibrium from multimers to monomers in cells.

Trypan blue staining and western blotting for the apoptotic marker, cleaved PARP, in M17D human neuroblastoma cells transfected with untagged aS showed multimer-abrogating variants (KLKEGV, KTKKGV, KTKEIV, KTKEGW) to be more neurotoxic than cells expressing wild-type aS or multimer-permissive variants (GTKEGV, KTEEGV, KTKEGR). Strikingly, multimer-abrogating variants yielded apparent aS-rich inclusions upon transfection in neuroblastoma cells and primary rat neurons as shown by immunocytochemistry. To characterize these aS inclusions, we tested the effects of candidate drugs that interfere with cellular vesicle trafficking, protein degradation and protein folding on inclusion formation by tetracycline-inducible aS expression in neuroblastoma cells. Unlike past studies, our analysis of aS inclusions are temporally sensitive due to controlling the multimer:monomer ratio via the induction of multimer-abrogating aS variants. In support of our overall model of PD pathogenesis, our data suggest strong neurotoxic effects from abolishing stable aS multimers in intact neural cells.

Disclosures: V. Von Saucken: None. **A.J. Newman:** None. **T. Bartels:** None. **U. Dettmer:** None. **D. Selkoe:** F. Consulting Fees (e.g., advisory boards); Prothena Biosciences.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.24/H21

Topic: C.03. Parkinson's Disease

Support: BMWi ZIM grant EP140389

Title: A functional phenotypic screening *in vitro* assay for novel Parkinson's drugs - comparing effects of human iPSC-derived dopaminergic neuronal networks and primary mouse midbrain cultures

Authors: *B. M. BADER, A.-M. PIELKA, C. EHNERT, K. JUEGELT, A. GRAMOWSKI-VOSS, O. H.-U. SCHROEDER;
NeuroProof GmbH, Rostock, Germany

Abstract: Human iPSC-derived neurons are promising tools to increase predictability, sensitivity and specificity of cell-based *in vitro* assays for drug discovery. Cell-based disease models are suitable for phenotypic screening which has led to the majority (7/8) of successfully launched CNS drugs in the last decade. Thus, our aim was to close the gap of functional phenotypic *in vitro* screening using human iPSC-derived neuronal networks of mesencephalic identity cultured on multiwell micro electrode arrays (mwMEA) for a toxin induced Parkinson's

screening model. We cultured human iPSC-derived post-mitotic dopaminergic neurons and recorded spontaneously activity during functional development of network communication patterns. We used a short pulse of the dopaminergic neuron-specific and sensitive toxin MPP+ to induce a strong functional but small morphological impairment. We further tested known neuroprotective compounds such as GDNF to prevent the functional impairment by single pre-treatment. Using multi-parametric data analysis of functional activity patterns, we calculated a read-out able to capture both, impairment and prevention of the neuronal network activity. We demonstrated that the used neuronal cultures produce robust spontaneous activity showing a functional maturation into a communicating network within 3 weeks *in vitro*. We further show that MPP+ induces significant functional effects which can be prevented by single GDNF treatment. We compare the results with those of primary mouse midbrain neuron/glia co-cultures using the same experimental design. In summary, our functional phenotypic *in vitro* screening platform with human iPSC-derived dopaminergic neurons growing on mwMEAs allows the robust and reproducible screening of novel leads or advanced drugs (repurposing) robust and reproducible screening of novel leads or advanced drugs (repurposing) for future therapies for Parkinson's disease.

Disclosures: **B.M. Bader:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. **A. Pielka:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. **C. Ehnert:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. **K. Juegelt:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. **A. Gramowski-Voss:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbH, Rostock, Germany. **O.H. Schroeder:** A. Employment/Salary (full or part-time):; NeuroProof GmbH, Rostock, Germany. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbHNeuroProof GmbH, Rostock, Germany.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.25/H22

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

Support: NIH Grant AA023172

Title: Effects of binge ethanol exposure on transient receptor potential melastatin 7 expression in brain microvascular endothelial cells of the HIV-1 transgenic rats

Authors: M. L. MACK[1], Y. WEI[1], M. D. LI[2], *S. L. CHANG[1];
[1]Inst. of NeuroImmune Pharmacol., Seton Hall Univ., South Orange, NJ; [2]Dept. of Psychiatry & Neurobehavioral Sci., Univ. of Virginia, Charlottesville, VA

Abstract: The HIV-1 transgenic (HIV-1Tg) rat was created with a *gag*- and *pol*-deleted HIV-1 viral genome under the control of the LTR viral promoter, and it persistently expresses 7 of the 9 HIV-1 genes. This rodent model mimics HIV-infected patients on combination anti-retroviral therapy (cART), who have controlled viral replication, but persistent HIV infection. The HIV-1Tg rat has also been used to study the effects of HIV-1 viral proteins on the response to addictive substances, including ethanol (EtOH). We previously demonstrated that the effects of binge exposure to EtOH are concentration dependent. EtOH exerts its physiological effects through a variety of target proteins, including transient receptor potential melastatin 7 channels (TRPM7). TRPM7 plays an essential role in maintaining cellular homeostasis of divalent ions, including Mg^{2+} and Ca^{2+}. We have recently shown that TRPM7 is highly expressed in primary cultures of mouse brain microvascular endothelial cells (BMVECs), the major cellular component of the blood-brain barrier (BBB). We found that EtOH modulates both protein and mRNA TRPM7 expression in a concentration-dependent manner, and that TRPM7 antagonists disrupt the integrity of an *in vitro* BBB model. In this study, rBMVECs were first isolated from both HIV-1Tg and F344 control rats at different ages, and then analyzed by flow cytometry for expression of intracellular TRPM7 protein. Our data showed that TRPM7 expression was lower in HIV-1Tg rats at 3-4 wks of age (25.6% TRPM7+) compared to that in the F344 control rats (44.1 %), whereas both F344 and HIV-1Tg rats expressed similar TRPM7 levels (42.2% and 40.4%, respectively) at 9 wks or older, indicating that the persistent presence of HIV-1 viral proteins delays TRPM7 expression in adolescents. Next, both 9 wk old HIV-1Tg and F344 rats were randomly assigned to receive saline or EtOH (2 g/kg/d, 3d) in either an 8% or 52% EtOH solution for 4 wks. Twenty four hours after the last treatment, rBMVECs were isolated from brain tissue and stained for intracellular TRPM7 protein. Binge exposure to EtOH for 4 wks decreased TRPM7 expression in rBMVECs of the F344 rats in a concentration-dependent manner; however, HIV-1Tg rats exhibited complete TRPM7 down-regulation when given either 8% or 52% EtOH. These results suggest that binge EtOH exposure completely blocks TRPM7 expression in adults, and could be one of the mechanisms underlying the damage to the BBB caused by repeated binge alcohol in HIV-1 patients, even with cART treatment.

Disclosures: M.L. Mack: None. **Y. Wei:** None. **M.D. Li:** None. **S.L. Chang:** None.

Poster

053. Neurodegeneration Drug Discovery: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 53.26/H23

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: Synthesis of high surface area gold nanoparticle for brain disease drug carrier

Authors: M. PARK, *S. CHUNG;
Dept. of Physiology, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: There are many types of brain diseases, such as an Alzheimer's Disease (AD) and Parkinson's Disease (PD). For the case of Alzheimer's Disease, it is caused mainly by beta-amyloid accumulation. To treat such disease, anthocyanin can be used to protect beta-amyloid accumulation. In case for Parkinson's Disease, it is caused by death of dopamine-generation cells in the midbrain region and many drugs are used for treatment. However, the possibility of the efficient drugs passing the BBB (Blood Brain Barrier) is low. Therefore, the use of drug carriers is an alternative method to treat brain disease. However, such treatment also requires several standards – ability to transport molecules across the BBB, biocompatibility, proper circulation time, and right particle size and shape. Based on such reasons, Gold nanoparticles satisfy as one of the best drug carriers for treating brain diseases. Here, we report method to synthesize gold nanoparticles, where size can be controlled with asymmetric shape, resulting in sufficient use as a drug carrier for treatment of brain diseases. To synthesize the gold nanoparticle, asymmetric gold nanoparticle's seed was used, also known as polyol process. Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) was used for metal precursor and ethylene glycol was used as a reducing agent, which donates electrons to gold ions ($Au^{3+} + 3e^- \rightarrow Au^0$). Finally, for surfactant, also called the Capping agent, poly (diallyldimethyl ammonium chloride) (PDDA) was used which has the ability to attach {111} plane, explained by the Miller Index. By mixing N, N-dimethylformamide (DMF) and Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), followed by 5 hour sonication, gold nanoparticles were synthesized. To check the nanoparticles, morphology, UV-Vis absorption, zeta potential and applications through Ussing chamber were checked. Through SEM (Scanning Electron Microscope) and TEM (Transmission Electron Microscopy), octahedral gold nanoparticles and asymmetric gold nanoparticles were successfully synthesized. Also, it was found that different experimental conditions, such as washing time, amount of gold ion's solution, concentration of DMF, and temperature, can affect the morphology of the gold nanoparticles. In addition through solution color observation, UV-Vis absorption, zeta potential and use of Ussing chamber, we confirmed the positive use of asymmetric gold nanoparticle's potential as use of nano drug carriers. Keywords: Asymmetric gold nanoparticle, Brain disease treatment, Drug carrier, Polyol process, Octahedral gold nanoparticle.

Disclosures: **M. Park:** None. **S. Chung:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.01/H24

Topic: C.19. Drug Discovery and Development

Title: Histamine H3 receptor inverse agonist, SUVN-G3031 produces procognitive effects without affecting sleep in preclinical animal models

Authors: *V. BENADE, S. DARIPELLI, J. THENTU, R. MEDAPATI, R. SUBRAMANIAN, V. MEKALA, A. SHINDE, L. KOTA, N. GANGADASARI, V. GOYAL, S. PANDEY, R. NIROGI;
Suven Life Sci. Ltd, Hyderabad, India

Abstract: SUVN-G3031, potent histamine 3 (H3) receptor inverse agonist is being developed for the treatment of cognitive deficits associated with Alzheimer's disease (AD). SUVN-G3031 is a lead molecule with hKi of 8.7 nM and has more than 100 fold selectivity against the related GPCRs. SUVN-G3031 exhibited desired pharmacokinetic properties and brain penetration. SUVN-G3031 blocked R-α-methylhistamine induced water intake and increased tele-methylhistamine levels in brain and cerebrospinal fluid. Treatment with SUVN-G3031 significantly reversed time induced memory deficit in novel object recognition test & T-maze task. It also reversed scopolamine induced memory deficit in Morris water maze task. Single dose oral administration resulted in H3 receptor occupancy up to 85% in rats and significantly raised acetylcholine and histamine levels in the cortex. At therapeutically effective doses, SUVN-G3031 produced no change in the sleep/ wake profile of rats. SUVN-G3031 was well tolerated in toxicity studies in animals with wide margin of safety, and was found to be non-genotoxic in bacterial reverse mutation assay and chromosomal aberration test in human lymphocytes. SUVN-G3031 produced procognitive effects in rats without affecting the sleep and it possess safety and desired pharmacokinetic profile for further development.

Disclosures: V. Benade: None. **S. Daripelli:** None. **J. Thentu:** None. **R. Medapati:** None. **R. Subramanian:** None. **V. Mekala:** None. **A. Shinde:** None. **L. Kota:** None. **N. Gangadasari:** None. **V. Goyal:** None. **S. Pandey:** None. **R. Nirogi:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.02/H25

Topic: C.19. Drug Discovery and Development

Support: Institut de Recherches

Title: Pharmacological profile of S 76892, a new ligand at nicotinic α7-subtype receptors

Authors: *C. LOUIS[1], N. ROGEZ[2], J.-Y. THOMAS[2], G. DAS DORES[2], A. HUGOT[2], M.-H. GANDON[2], V. BERTAINA-ANGLADE[5], A. KRAZEM[6], D. BÉRACOCHÉA[6], D. BERTRAND[7], D. RIMET[8], T. PILLOT[8], M. BERTRAND[3], I. BOTEZ[4], J.-M. FOURQUEZ[4], L. DANOBER[2], P. LESTAGE[2];
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Abstract: Ligands at α7 subtype receptors (α7-nAChRs) have been described as endowed with procognitive activity in preclinical and in clinical studies, both in Alzheimer's Disease and schizophrenia. Besides procognitive effects, action at this receptor might be of interest in relieving some deleterious actions induced by β-amyloid peptide, as already described in preclinical studies (eg : A-582941 (Hu et al 2008), S 24795 (Wang et al 2010), ABT-107 (Bitner et al 2010)). The aim of the present study was to characterize pharmacological properties of S 76892, a new ligand at α7-nAChR. S 76892 is a very weak partial agonist at rat α7-nAChR subtype, since it induced a very small inward current on Xenopus laevis oocytes injected with rat α7 mRNA. The current was blocked by α-bungarotoxin. As described for typical partial agonists (Briggs and McKenna, 1998), S 76892 decreased the ACh-induced current in a concentration dependant manner with an IC_{50} of 18 µM, confirming S 76892-mediated desensitization of the receptors. No agonistic activity of S 76892 was detected on Xenopus laevis oocytes injected with rat mRNA α4β2-nAChR. Potential protective effect of S 76892 on $A\beta_{1-42}$ peptide-induced toxicity on rat cortical cells culture was evaluated, S 24795 being used as an internal pharmacological reference. S 76892 had a very high permeability of the Blood-Brain-Barrier both in mice and in rats (Kp > 100). *In vivo*, S 76892 improved episodic-like memory in a novel object recognition test in mice and rats (3 mg/kg, p.o.). Furthermore, S 76892 reversed age-induced deficits in spatial contextual and serial discrimination task (0.1-0.3-1 mg/kg 9 administrations p.o.) in middle-aged mice. S 76892 improved spatial working memory as assessed by spontaneous alternation behavior on a T-maze in adult mice (0.03-0.1-0.3 mg/kg, i.p.). Besides its procognitive action, S 76892 also displayed neuroprotective activity (30 mg/kg

i.p.) in a model of delayed hippocampal neuronal death induced by a global transient ischemia in rats. S 76892 (1-3-10 mg/kg i.p.) did not induce wake-promoting effect as assessed by cortical EEG recordings in freely moving Wistar rats. Taken together, these results indicate that S 76892, on top of a potential counteractive action *in vitro* against Aβ-induced toxicity, enhances cognitive activity and is neuroprotective in rodents. It may therefore be of interest for pharmacological treatment of Alzheimer's disease.

Disclosures: **C. Louis:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **N. Rogez:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **J. Thomas:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **G. Das Dores:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **A. Hugot:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **M. Gandon:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **V. Bertaina-Anglade:** 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.; Biotrial. **A. Krazem:** 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.; CNRS/Université de Bordeaux. **D. Béracochéa:** 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.; CNRS/Université de Bordeaux. **D. Bertrand:** 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.; HiQScreen. **D. Rimet:** 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.; SynAging. **T. Pillot:** 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.; SynAging. **M. Bertrand:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **I. Botez:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **J. Fourquez:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **L. Danober:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **P. Lestage:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

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Program#/Poster#: 54.03/H26

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Support: The National Institute of Health (NIH)

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NSF Graduate Research Fellowship

Title: Step (striatal enriched protein tyrosine phosphatase) regulates tyrosine hydroxylase through bdnf signaling

Authors: *P. K. KURUP[1], J. XU[1], R. A. VIDEIRA[4], R. WICKAM[2], N. ADDY[2], G. BALTAZAR[4], A. NAIRN[2], P. LOMBROSO[1,2,3];
[1]Child Study Ctr., [2]Psychiatry, [3]Neurobio., Yale Univ. Sch. Med., New Haven, CT; [4]Hlth. Sci. Res. Center, CICS-UBI, Covilhã, Portugal

Abstract: (STriatal-Enriched protein tyrosine Phosphatase) is a protein tyrosine phosphatase implicated in several neuropsychiatric and neurodegenerative disorders. STEP is highly expressed in striatum, where it functions as a coincidence detector for converging dopamine and glutamate signals in medium spiny neurons. Here, we identify a novel of role STEP in dopaminergic neurons, where it acts as a link between neurotrophin and dopaminergic signaling. Our studies show STEP is expressed in midbrain dopaminergic neurons and its protein levels are regulated by the ubiquitin mediated proteasomal system through BDNF-TrkB signaling. Exposure of midbrain dopaminergic neurons to BDNF leads to degradation of STEP, sustained activation of ERK1/2 and increased phosphorylation of tyrosine hydroxylase (TH) at Ser^{31} (ERK1/2 site), which regulates TH activity and stability. In support of our findings, increased ERK1/2 activity in striatum from STEP KO mice is correlated with increased TH phosphorylation at Ser^{31} and increased TH protein levels, suggesting enhanced dopamine signaling. Our preliminary findings also suggest that STEP KO mice exhibit enhanced dopamine release compared to wild type mice. Our future studies are aimed at understanding the regulation of STEP by BDNF signaling and its role in TH activity, dopamine synthesis and release. These findings suggest a potential role of STEP in neuronal diseases where both BDNF and dopamine signaling are implicated. Funding: The National Institute of Health (NIH), and the Department of Defense (DOD), NSF Graduate Research Fellowship, CENTRO-07-ST24-FEDER-002015

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Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.04/H27

Topic: C.19. Drug Discovery and Development

Support: AG034140

Title: Novel small molecule triazolopyrimidine derivatives exhibit microtubule-stabilizing activity and represent potential therapeutic candidates for Alzheimer’s disease and related tauopathies

Authors: J. KOVALEVICH[1], A.-S. CORNEC[2], Y. YAO[1], M. JAMES[1], A. CROWE[1], V. M. Y. LEE[1], J. Q. TROJANOWSKI[1], A. B. SMITH, III[2], C. BALLATORE[2], *K. R. BRUNDEN[1]; [1]Ctr. Neurodegen Res., Univ. Pennsylvania, Philadelphia, PA; [2]Chem., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Alzheimer’s disease (AD) and related tauopathies are characterized by hyperphosphorylation and aggregation of the microtubule (MT)-associated protein, tau. Through incompletely understood mechanisms, hyperphosphorylation of tau induces its dissociation from MTs, where it normally plays an integral role in MT stability and facilitation of axonal transport in neurons. Loss of tau function leads to MT hyperdynamicity and disrupted transport of critical cellular proteins, likely leading to neuronal dysfunction and cognitive decline. Therefore, compensation for tau loss-of-function through introduction of MT-stabilizing compounds might normalize the altered MT dynamics that accompany tauopathies and serves as a viable therapeutic approach in the treatment of AD and related tauopathies. In this context, we have generated a number of brain-penetrant small molecule triazolopyrimidine derivatives that exhibit MT-stabilizing activity in both proliferating cells and in rat primary cortical neurons, as assessed by post-translational modifications of tubulin (i.e.; acetylation and detyrosination) that are indicative of MT stabilization. Interestingly, although highly similar in structure, these small molecules can be subdivided into two classes based on their *in vitro* behavior. One set of compounds, deemed “Class 1” molecules, increase markers of stable MTs in dividing cells and

in primary neurons without affecting total alpha- or beta-tubulin levels or significantly altering MT morphology at effective doses. The structurally related "Class 2" molecules increase markers of stable MTs at moderate doses in QBI-293 cells (a HEK-293 cell derivative) but not in primary neurons, and appear to induce degradation of alpha- and beta-tubulin across multiple cell types. Furthermore, in QBI-293 cells, Class 2 compounds lose efficacy in inducing post-translational modifications of tubulin indicative of stabilization at high doses. Although Class 2 compounds appear to possess MT-stabilizing activity based on activity in the acetyl-tubulin assay at moderate doses, their substantial negative impact on total tubulin levels likely preclude them from providing any therapeutic benefit under pathological tau conditions. Therefore, studies to further characterize the molecular mechanisms underlying the effects of Class 1 compounds on MT stability and axonal transport, as well as the potential for therapeutic use through compensation for tau deficits in AD, are currently underway.

Disclosures: J. Kovalevich: None. **A. Cornec:** None. **Y. Yao:** None. **M. James:** None. **A. Crowe:** None. **V.M.Y. Lee:** None. **J.Q. Trojanowski:** None. **A.B. Smith:** None. **C. Ballatore:** None. **K.R. Brunden:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.05/H28

Topic: C.19. Drug Discovery and Development

Title: Clinical pharmacology of the 5-HT6 antagonist, RVT-101: summary of phase 1 studies

Authors: I. LOMBARDO[1], *L. FRIEDHOFF[1], S. PISCITELLI[2], G. RAMASWAMY[3]; [1]Axovant Sciences, Inc, New York, NY; [2]Roivant Sciences, Inc, New York, NY; [3]Axovant Sciences, Inc., New York, NY

Abstract: Background: RVT-101 (formerly known as SB-742457) is an orally administered, 55-HT6) serotonin receptor antagonist that promotes the release of acetylcholine and other neurotransmitters. RVT-101 is being evaluated in Alzheimer's disease (AD) as an adjunctive therapy to the cholinesterase inhibitor, donepezil. Methods: Nine studies in 225 healthy subjects were completed. These include single-and repeat-dose studies, positron emission tomography (PET) studies (data not presented here), and drug interaction studies. Population PK analyses were also conducted using data from Phase 2 studies. Healthy adults have received single doses ranging from 2 to 175 mg. Multiple doses up to 35 mg daily for 28 days in healthy elderly subjects and up to 50 mg daily for 13 days in younger adult subjects have been administered.

Results: RVT-101 demonstrates linear pharmacokinetics (PK) over the dosage range studied. The average time to maximal concentration was 4 to 7 hours and the half-life was approximately 30 hours. On repeat dosing, steady state was reached within 7 days. The PK were not affected by administration with a high fat meal. No major age or gender differences in PK have been observed, and the PK in subjects with AD was similar to that in healthy volunteers. A CYP450 probe interaction study showed RVT-101 has no significant effects on 3A4, 2D6 and 1A2 metabolic pathways. RVT-101 exhibits modest inhibitory effects on CYP2C9; the international normalized ratio (INR) was 23% higher when warfarin was co-administered with RVT-101 compared with co-administration with placebo. Studies also demonstrated that RVT-101 did not significantly alter the exposure of donepezil or risperidone. Conclusion: Phase 1 studies demonstrate that RVT-101 possesses a number of favorable properties for use in the AD population including once daily dosing, lack of food effect, and low potential for drug interactions.

Disclosures: I. Lombardo: A. Employment/Salary (full or part-time):; Axovant Sciences, Inc. **L. Friedhoff:** A. Employment/Salary (full or part-time):; Axovant Sciences, Inc. **S. Piscitelli:** A. Employment/Salary (full or part-time):; Roivant Sciences, Inc. **G. Ramaswamy:** A. Employment/Salary (full or part-time):; Axovant Sciences, Inc..

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.06/H29

Topic: C.19. Drug Discovery and Development

Support: NIMH

Title: Effects of inhibiting STriatal-Enriched protein tyrosine Phosphatase (STEP) on dendritic morphology in mice with Alzheimer's disease

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[2]Child Study Ctr., [3]Chem., [4]Psychiatry, [5]Neurobio., [1]Yale Univ., New Haven, CT

Abstract: STEP (STriatal-Enriched protein tyrosine Phosphatase) is a neuron-specific phosphatase that is overactive in several neuropsychiatric conditions including Alzheimer's disease (AD). Alzheimer's disease presents with reduced dendritic complexity, which is thought to cause cognitive deficits through alterations in synaptic connectivity. Genetic reduction of

STEP resulted in significant behavioral improvements in 3xTg AD mice, and validated STEP as a target for drug discovery. We now have a STEP inhibitor, TC-2153, which is a potent and specific inhibitor of STEP. STEP inhibition by TC-2153 is achieved through oxidative attack of the catalytic cysteine residue in the phosphatase domain of STEP. Administration of TC-2153 also improved the cognitive functions of 3xTg mice in various behavioral paradigms. Here we present the effects of pharmacological inhibition of STEP on the dendritic morphology in 3xTg AD mice. Using Golgi Cox staining, dendritic morphology was analyzed in 6-month old WT and AD mice treated with a vehicle, then compared to WT and AD mice treated with TC-2153 for two weeks. Due to improvements in the behavior and dendritic morphology of AD mice when treated with a STEP inhibitor, we consider STEP a novel target for the treatment of a range of psychiatric disorders including AD.

Disclosures: J.B. Benedict: None. **M. Chatterjee:** None. **J. Ellmann:** None. **A. Nairn:** None. **P. Lombroso:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

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Program#/Poster#: 54.07/H30

Topic: C.19. Drug Discovery and Development

Title: Selective sphingosine-1-phosphate receptor 5 agonists can modulate lipid content in the brain and thereby potentially treat neurodegenerative disorders

Authors: *E. VAN DER KAM[1], S. C. TURNER[2], M. OCHSE[2], J. VAN BERGEIJK[2], R. MUELLER[2], M. MEZLER[3], K. HEMPEL[4], A. HOBSON[6], C. M. HARRIS[6], A. BESPALOV[2], A. HAHN[2], B. RENDENBACH-MUELLER[5];
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Abstract: Sphingosine-1-phosphate (S1P) plays an important role as a regulator of signal transduction, cellular plasticity, cell proliferation, membrane stability/BBB integrity, and is proposed to modulate the ceramide-S1P and cholesterol homeostasis. S1P exerts these actions through G protein-coupled receptors, such as the brain-preferred S1P5 receptor. Given these actions, S1P5 agonism could potentially be a beneficial treatment for neurodegenerative disorders. AbbVie has recently developed highly selective S1P5 receptor agonists, such as A-

971432. A-971432 has an EC50 of 10 nM on the hS1P5 receptor with a large selectivity window. The compound shows an excellent *in vivo* PK profile with good oral bio-availability (60% in mice, 100% in rat, 92% in dogs), half-life (7-9h), and brain penetration (B/P ~0.2). Moreover, the compound was well tolerated in a 14d Sprague-Dawley rat exploratory toxicity study up to 100 mg/kg bw/day and with favorable profile in cardiovascular examination. As such, this compound was used to test the hypothesis that selective S1P5 agonists will a) reduce CNS, but not peripheral lipid content, b) improve cognition, and c) change disease progression as assessed in a model mimicking key features of AD. A-971432 was examined in models of age-related cognitive decline, such as the spontaneous alternation T-maze assay, the Morris Water Maze (MWM), and the Object Recognition (ORT) task in aged (12+ months or 18+ months) mice and rats. Sub-acute treatment (≥7 days) in either T-maze (0.03 mg/kg - 3 mg/kg) or MWM/ORT (0.1 and 0.5 mg/kg) fully reversed age-related cognitive deficits with a minimal effective dose of 0.1 mg/kg (40 ng/mL). Concomitantly, A-971432 normalizes the age-related CNS sphingolipid imbalance without affecting plasma levels. Finally, a single dose was examined in the murine model of Niemann Pick C disease (NPC). NPC (endosomal-lysosomal storage disease) animals display AD-like pathology such as Aβ and Tau accumulation. A-971432 (1 mg/kg) was able to improve the behavioral phenotype (dystonia, motor impairment), promote survival, normalized CNS sphingolipid content, and normalized Aβ levels in the CSF. The data, together with the cognition and lipid modulation findings indicate that S1P5 agonism provides an innovative mechanism for the potential treatment of neurodegenerative disorders such as AD and lysosomal storage disorders such as Niemann Pick C. Disclosures: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Disclosures: E. Van Der Kam: A. Employment/Salary (full or part-time):; AbbVie Deutschland GmbH & Co KG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie Inc. **S.C. Turner:** A. Employment/Salary (full or part-time):; AbbVie Deutschland GmbH & CO KG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie Inc. **M. Ochse:** A. Employment/Salary (full or part-time):; AbbVie Deutschland GmbH & CO KG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie Inc. **J. van Bergeijk:** A. Employment/Salary (full or part-time):; AbbVie Deutschland GmbH & CO KG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie Inc. **R. Mueller:** A. Employment/Salary (full or part-time):; AbbVie Deutschland GmbH & CO KG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie Inc. **M. Mezler:** A. Employment/Salary (full or part-time):; AbbVie Deutschland GmbH & CO KG. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AbbVie Inc. **K. Hempel:** A. Employment/Salary (full or part-time):; AbbVie

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Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.08/H31

Topic: C.19. Drug Discovery and Development

Title: SUVN-D4010: Novel 5-HT4 receptor partial agonist for the treatment of Alzheimer's disease

Authors: ***N. MUDDANA**, R. SUBRAMANIAN, R. MEDAPATI, R. ABRAHAM, V. BENADE, R. PALACHARLA, A. MANOHARAN, V. GOYAL, S. PANDEY, M. RASHEED, S. RAVELLA, R. NIROGI;
Suven Life Sci., Hyderabad, India

Abstract: SUVN-D4010 is a potent, selective and orally bioavailable 5-HT4 partial agonist being developed for the treatment of Alzheimer's disease. The procognitive properties of SUVN-D4010 were characterized in animal models of cognition like object recognition task, radial arm maze task and fear conditioning assay. The effect on the cholinergic neurotransmission was studied using brain microdialysis technique. *In vivo* receptor binding profile was measured using non-radiolabeled tracer in rats. The effect of SUVN-D4010 on the toxic β-amyloid and the

neuroprotective sAPPα were evaluated in the preclinical species using ELISA kits. Safety, general toxicity and mutagenic potential of SUVN-D4010 were evaluated in rodents/non rodents and *in vitro* models. SUVN-D4010 improved the episodic memory deficits in object recognition task. It also reversed the working and emotional memory deficits induced by scopolamine in radial arm maze task and fear conditioning assay. Oral administration of SUVN-D4010 significantly increased the brain acetylcholine levels. SUVN-D4010 also potentiated the pharmacological effects of donepezil. The effect on the cognition and cholinergic neurotransmission were blocked by GR 125478, a selective 5-HT4 antagonist. SUVN-D4010 showed significant 5-HT4 receptor occupancy at pharmacologically effective doses. A significant increase in cortical sAPPα and decrease in amyloid-β protein levels was also seen. SUVN-D4010 was well tolerated in animal toxicity studies and did not show any mutagenic potential. SUVN-D4010 is a novel, potent, selective, orally bioavailable, efficacious and a safe 5-HT4 receptor partial agonist. IND enabling GLP safety studies have been completed. US IND filing is in progress.

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Poster

054. Neurodegeneration Drug Discovery

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Topic: C.19. Drug Discovery and Development

Support: NIH 1R15Ns072879-01A1

Title: Pharmacological inhibition of striatal-enriched protein tyrosine phosphatase (STEP) with TC-2153 attenuates seizure frequency and severity in the pilocarpine model of temporal lobe epilepsy

Authors: *D. B. LAWRENCE[1], R. M. SAMPLES[1], F. A. HARRSCH[1], E. S. WEISS[1], M. R. PELTON[1], M. VAN ZANDT[1], M. CHATTERJEE[2], T. BAGULEY[3], J. ELLMAN[3], A. C. NAIRN[3], P. J. LOMBROSO[2,4,5], J. R. NAEGELE[1];
[1]Dept. of Biol., Wesleyan Univ., Middletown, CT; [2]Child Study Ctr., [3]Dept. of Chem., [4]Psychiatry, [5]Neurobio., Yale Univ., New Haven, CT

Abstract: Striatal-Enriched Protein Tyrosine Phosphatase (STEP) levels are elevated in several neurological disorders, including Alzheimer's disease and schizophrenia. STEP deficiency caused by gene knockout or pharmacological inhibition of STEP with TC-2153, a STEP inhibitor, ameliorates cognitive deficits in these disorders. A role for STEP in epilepsy is implicated by prior studies showing that STEP knockout mice exhibit increased seizure thresholds in the pilocarpine model of temporal lobe epilepsy (TLE). The present study was conducted to determine whether STEP inactivation with TC-2153 prevents or attenuates seizures. Following pilocarpine induced status epilepticus (SE) in C57BL/6 mice (Harlan Laboratories,) TC-2153 was injected intraperitoneally every day for two weeks at doses of 0, 5, or 10 mg/kg. Seizure activity from 40 mice was monitored with continuous video-EEG (vEEG) for varying periods from 21 days to 85 days after SE. vEEG analyses were carried out on 27 mice from post SE days 40-60 to quantify seizure frequency, severity, and duration. Mice receiving TC-2153 injections showed significantly reduced seizure frequency and severity. Studies are underway to examine seizure-induced changes in STEP levels and the effects of TC-2153 on STEP substrate phosphorylation after SE. Our preliminary findings suggest a role for STEP in epileptogenesis and potential therapeutic benefits of STEP inhibition for the treatment of TLE.

Disclosures: D.B. Lawrence: None. **R.M. Samples:** None. **F.A. Harrsch:** None. **E.S. Weiss:** None. **M.R. Pelton:** None. **M. Van Zandt:** None. **M. Chatterjee:** None. **T. Baguley:** None. **J. Ellman:** None. **A.C. Nairn:** None. **P.J. Lombroso:** None. **J.R. Naegele:** None.

Poster

054. Neurodegeneration Drug Discovery

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.19. Drug Discovery and Development

Support: NIH Grant R00AT004197

Title: Development of a novel and robust pharmacological model of okadaic acid-induced Alzheimer's disease in zebrafish

Authors: S. NADA[1], F. E. WILLIAMS[2], *Z. A. SHAH[3];
[1]Univ. of Toledo, Toledo, OH; [2]Pharmacol., [3]Medicinal & Biol. Chem., Univ. of Toledo Col. of Pharm. and Pharmaceut. Sci., Toledo, OH

Abstract: Alzheimer's disease (AD) is the leading neurodegenerative disorder that incapacitates the elderly population all over the world. Most of the experimental models for AD are transgenic

or pharmacological in nature and do not simulate the pathophysiology in entirety. In the present study we intended to develop a pharmacologically induced AD using economically affordable species that will recapitulate most of the phenotypes of the disease. The pharmacological agent, okadaic acid (OKA) used in this study is the best pharmacological agent that has already been shown to work in other species. In this model the expression profile of phosphorylation of GSK-3α/β, Aβ, p-Tau and Tau protein and senile plaque formation in zebrafish brain were significantly increased with the exposure of increasing concentration of OKA and these represent the majority of the hallmarks of AD pathophysiology. The observed morphological changes were also accompanied by the learning and memory deficits which are important physiological components in AD pathophysiology. Zebrafish disease models are gaining popularity mostly due to their economic cost and relevance to human disease pathophysiology. Existing pharmacological methods of inducing AD are not adequately developed in this species and do not represent all the features of the disease as was observed in OKA-induced AD model. OKA-induced AD in zebrafish can become a robust model to study drug discovery for AD or unravel the molecular mechanisms underlying the complex pathophysiology that leads to AD using relatively economical species.

Disclosures: **S. Nada:** None. **F.E. Williams:** None. **Z.A. Shah:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.11/H34

Topic: C.19. Drug Discovery and Development

Support: NIH Grant 1F32AG039220

NIH Grant 1K99AG048222

UCSF Alzheimer's Disease Research Center

Alan Kaganov Scholarship

S.D. Bechtel, Jr. Foundation

MetLife Foundation

Title: Adenosine A2A receptor expression in human brain and beneficial effects of A2A receptor blockade in aging and models of neurodegenerative disease

Authors: *A. G. ORR[1,2], K. HO[1], A. LUNDQUIST[1], G. SHLAGER[1], S. LEE[1], D. H. KIM[1], X. WANG[1], W. GUO[1], G.-Q. YU[1], E. MASLIAH[3,4], W. W. SEELEY[2,5], L. MUCKE[1,2]; [1]Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [2]Dept. of Neurol., Univ. of California, San Francisco, CA; [3]Dept. of Neurosci., [4]Dept. of Pathology, Univ. of California, San Diego, CA; [5]Dept. of Pathology, Univ. of California, San Francisco, CA

Abstract: Adenosine receptor signaling regulates neural function and may play a role in neurodegenerative disorders. We previously reported that hippocampal astrocytes in humans with sporadic Alzheimer's disease (AD) had increased levels of the adenosine receptor A2A and these increases correlated with astrogliosis and tau pathology (Orr et al., 2015, Nat Neurosci). In the same study, we demonstrated that conditional genetic ablation of astrocytic A2A receptors prevented memory loss in mice with or without amyloid plaque deposition, suggesting that these receptors regulate cognitive function. In the present study, we further investigated the expression of A2A receptors in postmortem human brain tissues from aging nondemented controls and cases with sporadic AD, dementia with Lewy bodies (DLB), or frontotemporal lobar degeneration (FTLD). Early data show that astrocytic A2A receptor levels are increased in the hippocampal formation, but not frontal cortex, of cases with AD, DLB or FTLD, suggesting a brain region-specific response of astrocytes that could contribute to memory loss in these diverse neurodegenerative conditions. Together, our findings also imply that blocking astrocytic A2A receptors could be a therapeutic strategy for memory enhancement. However, A2A receptors are also highly expressed by striatal medium spiny neurons and known to regulate motor function. Therefore, we tested the effects of global pharmacological blockade of A2A receptors on cognitive and motor functions in aging animals. After conducting preliminary pharmacokinetic analyses, we treated aging nontransgenic (NTG) and transgenic mice expressing human amyloid precursor protein (hAPP) with istradefylline, a potent and selective A2A receptor antagonist. We found that administration of high doses of istradefylline induced hyperactivity in the open field, but did not affect context habituation, in NTG and hAPP mice. In contrast, lower doses of istradefylline enhanced context habituation in NTG and hAPP mice without inducing prolonged hyperactivity or anxiety. Low doses of istradefylline also enhanced motor performance in the rotarod test, suggesting that, within an appropriate therapeutic window, A2A receptor blockade can enhance both cognitive and motor functions in aging animals. Our ongoing neuropathological and preclinical studies may guide the development of therapeutic strategies for AD and other neurodegenerative disorders that involve aberrant A2A receptor activity.

Disclosures: A.G. Orr: None. **K. Ho:** None. **A. Lundquist:** None. **G. Shlager:** None. **S. Lee:** None. **D.H. Kim:** None. **X. Wang:** None. **W. Guo:** None. **G. Yu:** None. **E. Masliah:** None. **W.W. Seeley:** None. **L. Mucke:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.12/H35

Topic: C.19. Drug Discovery and Development

Title: Safety, tolerability and pharmacokinetics of a potent and selective H3 receptor inverse agonist, SUVN-G3031 following single and multiple ascending doses in healthy adult subjects

Authors: *G. BHYRAPUNENI, K. MUDIGONDA, K. PENTA, N. MUDDANNA, R. PALACHARLA, P. JAYARAJAN, R. ABRAHAM, R. SUBRAMANIAN, V. GOYAL, S. PANDEY, R. BOGGAVARAPU, D. AJJALA, A. SHINDE, R. NIROGI;
Suven Life Sci., Hyderabad, India

Abstract: SUVN-G3031, a potent and selective histamine 3 (H3) receptor inverse agonist is being developed for the treatment of cognitive deficits associated with Alzheimer's disease (AD). SUVN-G3031 demonstrated cognitive enhancement and relevant neurochemical changes without affecting the sleep in rodent models. SUVN-G3031 was studied in a single-center, multi-faceted, phase 1 clinical trial (US IND) to evaluate its safety, tolerability, and pharmacokinetics after single and multiple ascending doses in healthy young adult subjects. SUVN-G3031 was quantified in plasma using a validated LC-MS/MS method. SUVN-G3031 was tolerated up to 20 mg/day following single oral administration in healthy adult subjects. There were no clinically relevant or serious adverse events observed at any of the doses tested. During multiple ascending dose studies, SUVN-G3031 has shown a favorable pharmacokinetic profile. SUVN-G3031 achieved the projected efficacy concentrations and attained steady state from seven days in the tested population. SUVN-G3031 is well tolerated in humans with adequate plasma exposure for efficacy and favorable pharmacokinetics suitable for once a day oral administration.

Disclosures: G. Bhyrapuneni: None. **K. Mudigonda:** None. **K. Penta:** None. **N. Muddanna:** None. **R. Palacharla:** None. **P. Jayarajan:** None. **R. Abraham:** None. **R. Subramanian:** None. **V. Goyal:** None. **S. Pandey:** None. **R. Boggavarapu:** None. **D. Ajjala:** None. **A. Shinde:** None. **R. Nirogi:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.13/H36

Topic: C.19. Drug Discovery and Development

Support: FRAXA

Title: Inhibitors of STEP as a novel treatment of fragile-x-syndrome

Authors: *M. CHATTERJEE[1], J. KWON[1], J. BENEDICT[1], E. FOSCUE[1], T. BAGULEY[2], J. ELLMANN[2], A. NAIRN[3], P. LOMBROSO[1,3,4];
[1]Child Study Ctr., [2]Chem., [3]Psychiatry, [4]Neurobio., Yale, New Haven, CT

Abstract: STEP is a brain specific phosphatase has recently emerged as a promising target in various neuropsychiatric condition including Alzheimer's disease, Parkinson's disease, schizophrenia and fragile-X syndrome. Fragile X syndrome (FXS), an X-linked condition caused by an increase in the number of CGG repeats located in the first exon of the Fragile X mental retardation 1 (FMR1) gene, which encodes the RNA-binding protein FMRP. FMRP acts as a translational silencer of mRNAs that encode several synaptic proteins. In the absence of FMRP, translation of some of these mRNAs is upregulated and one such message that associates with FMRP is STEP (STriatal-Enriched protein tyrosine Phosphatase) mRNA. STEP is known to be downstream of the Fmr1 protein and Fmr1 suppresses the translation of STEP. Absence of Fmr1 gene in mice leads to overexpression of STEP, dephosphorylating its key substrates GluN2B, ERK and Pyk 2 mainly involved in synaptic strengthening. Genetic reduction of STEP in Fmr1 KO mice showed improvements in behavioral abnormalities commonly found in fragile-X mice. Our recent focus was on developing a STEP Inhibitor, which can rescue these abnormalities in KO mice. TC-2153, a potent STEP inhibitor has been found to inhibit STEP in WT mice and is able to rescue memory related abnormalities in AD mice model. Our goal was to test whether TC-2153 rescues the behavioral abnormalities in fragile X mouse model similar to genetic reduction of STEP. Further, we will also look at the effect of this inhibitor in rescuing abnormalities in dendritic spine density and morphology in cell based assays. Funding: FRAXA

Disclosures: **M. Chatterjee:** None. **J. Kwon:** None. **J. Benedict:** None. **E. Foscue:** None. **T. Baguley:** None. **J. Ellmann:** None. **A. Nairn:** None. **P. Lombroso:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.14/H37

Topic: C.19. Drug Discovery and Development

Support: NIMH

Title: Exercise regulates STEP (STriatal-Enriched protein tyrosine Phosphatase) through BDNF signaling

Authors: *M. POWELL[1], D. GHOSH[1], E. P. FOSCUE[1], A. C. NAIRN[2], P. J. LOMBROSO[3]; [1]Yale Child Study Ctr., [2]Psychiatry, [3]Yale Child Study Center, Neurobiology, Psychiatry, Yale Univ., New Haven, CT

Abstract: Exercise has been widely accepted to increase general health, but over the last fifteen years, interest in the strengthening effects of exercise on specific brain functions has grown. Brain-derived neurotrophic factor (BDNF) is upregulated by exercise and correlated with synaptic plasticity and cell genesis, growth, and survival. It has been suggested that the majority of exercise-induced neuronal strengthening takes place through N-methyl-D-aspartate (NMDA) receptor glutamatergic transmission, as lack of the key NR2A subunit of the NMDA receptor prevents exercise from increasing neurogenesis or BDNF levels in the brain. The NMDA receptor is necessary for improving memory and learning, enabling neurons to modify themselves in accordance with past stimulations. A regulator of NMDA receptor trafficking is a tyrosine phosphatase called striatal-enriched protein tyrosine phosphatase, or STEP. STEP de-phosphorylates many substrates linked with brain maintenance and degradation, including the NR2B subunit of the NMDA receptor (GluN2B), which results in internalization of the receptor within the neuron. Through this mechanism, STEP opposes the development of synaptic strengthening and is linked to various neuropsychiatric disorders, including Alzheimer's disease, fragile X syndrome, and schizophrenia. Previous work has shown that STEP levels have an inverse relationship with BDNF. Our findings demonstrate that exercised animals have lower levels of STEP as compared to sedentary age-matched controls. In addition, experiments using the Y maze behavior task revealed that exercised animals improved their working memories over sedentary animals, and experiments using the novel object recognition behavior task added that exercised STEP knockout (KO) animals, though their cognition was improved over their wild-type counterparts, did not exceed sedentary STEP KO animals on the task. These findings, taken together with an understanding of STEP regulatory mechanisms, have led to the hypothesis that exercise leads to elevated BDNF resulting in STEP degradation through the ubiquitinate proteasome pathway. Lower levels of STEP leads to increased phospho-extracellular signal-related kinase (pERK1/2) and phospho-NR2B (pGluN2B), with improvements in cognition and synaptic strengthening. We seek to better understand this pathway in the hope that it may eventually become a target for therapeutics seeking to replicate the beneficial effects of exercise on the brain.

Disclosures: M. Powell: None. **D. Ghosh:** None. **E.P. Foscue:** None. **A.C. Nairn:** None. **P.J. Lombroso:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.15/H38

Topic: C.19. Drug Discovery and Development

Support: Michael J. Fox Foundation Grant

Title: Discovery and clinical development of npt088, a general amyloid interaction motif (gaim)-immunoglobulin fusion protein

Authors: *J. M. LEVENSON[1], K. S. GANNON[2], J. C. CARROLL[2], S. SCHROETER[2], V. CULLEN[2], E. ASP[3], C. CHUNG[3], M. GARTNER[3], M. LULU[3], M. PROSCHITSKY[3], H. TSUBERY[3], R. KRISHNAN[3], E. ROCKENSTEIN[7], E. MASLIAH[7], M. NADEEM[8], E. J. MUFSON[8], M. GRAY[4], M. GRUNDMAN[4], R. BALES[5], J. WRIGHT[6], B. SOLOMON[9], F. HEFTI[1], R. FISHER[3];
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Abstract: Alzheimer's disease (AD) and other neurodegenerative disorders are associated with the accumulation of aggregates of multiple species of misfolded proteins, such as amyloid-β (Aβ), tau, and α-synuclein. The need to target multiple types of protein aggregates in a single disease suggests the need for therapeutic strategies that target aggregates independent of primary protein sequence. We isolated a protein motif from bacteriophage M13 that binds and remodels multiple misfolded proteins that assume an amyloid conformation including Aβ, tau, and α-synuclein. We call this motif the General Amyloid Interaction Motif (GAIM). NPT088, our development candidate, is a human immunoglobulin (huIgG1Fc) fusion protein that displays two copies of GAIM. We have shown that NPT088 has uniquely broad activities, both *in vitro* and *in vivo*, against multiple neuropathological aggregates, making it a novel candidate for treating AD. NPT088 specifically and potently binds amyloid fibers of Aβ, tau and α-synuclein, but does not bind the corresponding monomers or native protein assemblies. NPT088 binds Aβ oligomers, prevents Aβ and tau aggregation, and protects differentiated N2a cells from Aβ oligomer-induced cytotoxicity. NPT088 recognizes Aβ aggregates from brains of aged Tg2576 mice. NPT088 is efficacious in transgenic AD and Parkinson's disease mouse models. In the Tg2576 hAPP model, NPT088 significantly improves cognition, reduces brain Aβ(1-42) and Aβ plaque load, and reduces Aβ in cerebral spinal fluid. In rTg4510 tau mice, NPT088 significantly improves cognition and reduces levels of phospho-tau associated with neuropathology. In

mThy1-Hα-synuclein mice, NPT088 significantly reduces proteinase K-resistant α-synuclein and increases tyrosine hydroxylase levels. NPT088 has successfully completed pilot rat and monkey safety studies. These results demonstrate that NPT088 is a first-in-class therapeutic candidate for AD and other neurodegenerative diseases that targets misfolded proteins, including aggregates of Aβ, tau and α-synuclein. Following IND filing in 4Q2015, NPT088 will be tested for safety in healthy volunteers and then for proof of activity in AD patients by measuring reduction of PET amyloid markers.

Disclosures: J.M. Levenson: A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **K.S. Gannon:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurophage Pharmaceuticals. **J.C. Carroll:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **S. Schroeter:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **V. Cullen:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **E. Asp:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **C. Chung:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **M. Gartner:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **M. Lulu:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **M. Proschitsky:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **H. Tsubery:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **R. Krishnan:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **E. Rockenstein:** F. Consulting Fees (e.g., advisory boards); NeuroPhage Pharmaceuticals. **E. Masliah:** 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.; NeuroPhage Pharmaceuticals. **M. Nadeem:** 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.; NeuroPhage Pharmaceuticals. **E.J. Mufson:** 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.; NeuroPhage Pharmaceuticals. F. Consulting Fees (e.g., advisory boards); NeuroPhage Pharmaceuticals. **M. Gray:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **M. Grundman:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **R. Bales:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **J. Wright:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **B. Solomon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. F. Consulting Fees (e.g., advisory boards); NeuroPhage Pharmaceuticals. **F. Hefti:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals. **R. Fisher:** A. Employment/Salary (full or part-time):; NeuroPhage Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroPhage Pharmaceuticals.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.16/H39

Topic: C.19. Drug Discovery and Development

Support: Michael J Fox Foundation for Parkinson's Disease Research LEAPS Award

Title: PD-61-W3 (Synuclere™) reduces and detoxifies alpha-synuclein aggregates and improves motor dysfunction: Development of a potential novel Parkinson's disease-modifying therapeutic

Authors: *L. A. ESPOSITO, K. L. HANSON, J. CUMMINGS, M.-C. YADON, T. M. CHONG, T. LAKE, Q. HU, J. CAM, A. D. SNOW;
ProteoTech Inc, Kirkland, WA

Abstract: PD-61-W3 (*Synuclere*™) is an organic proprietary small molecule that inhibits the formation of toxic alpha-synuclein aggregates at substoichiometric proportions and rapidly reduces and detoxifies pre-formed alpha-synuclein aggregates, a central pathogenic component in Parkinson's Disease (PD). PD-61-W3 efficacy and mechanism of action was established in a comprehensive set of *in vitro,* cell-based and *in vivo* (animal model) studies. Most importantly, PD-61-W3 efficacy was demonstrated in independent studies using PD-relevant Thy-1 human wild-type alpha-synuclein transgenic mice (Line 61). These studies showed that PD-61-W3 is well-tolerated *in vivo* and that PD-61-W3 targets brain alpha-synuclein as evidenced by marked reductions in alpha-synuclein aggregates in the substantia nigra, cortex and hippocampus, following 3-month treatment of younger mice (45-90% reductions) and 6-month treatment of older mice (79-91% reductions). Western blot analysis of brain extracts showed PD-61-W3 treatment significantly reduced soluble alpha-synuclein oligomers (by 72%). Reduced alpha-synuclein aggregates in PD-61-W3-treated transgenic mice were accompanied by improved motor performance on the challenging beam traversal and pole tests. The primary backup molecule, PD-61-F2, (an analog of PD-61-W3) also significantly reduced brain -synuclein aggregates (by 62%-96%). Additionally, drugability studies demonstrate that PD-61-W3 exhibits good properties characteristic of a CNS drug, including micromolar exposure in brain, plasma and CSF following a single subcutaneous injection in mice at a therapeutic dose level, no significant off-target binding to a panel of brain receptors, transporters or ion channels, no significant CYP450 inhibition, and good chemical stability. Furthermore, PD-61-W3 is non-mutagenic in the Ames test at dose levels up to 5000 micrograms per plate. In conclusion, PD-61-W3 safely and effectively reduced alpha-synuclein aggregation, improved motor function, and is being developed as a disease modifying therapeutic for Parkinson's Disease and related synucleinopathies. Funded by ProteoTech and a LEAPS Award from the Michael J. Fox Foundation for Parkinson's Disease Research.

Disclosures: **L.A. Esposito:** A. Employment/Salary (full or part-time):; ProteoTech Inc. **K.L. Hanson:** A. Employment/Salary (full or part-time):; ProteoTech Inc. **J. Cummings:** A. Employment/Salary (full or part-time):; ProteoTech Inc. **M. Yadon:** A. Employment/Salary (full or part-time):; ProteoTech Inc. **T.M. Chong:** A. Employment/Salary (full or part-time):; ProteoTech Inc. **T. Lake:** A. Employment/Salary (full or part-time):; ProteoTech Inc. **Q. Hu:** A.

Employment/Salary (full or part-time):; ProteoTech Inc. **J. Cam:** A. Employment/Salary (full or part-time):; ProteoTech Inc. **A.D. Snow:** A. Employment/Salary (full or part-time):; ProteoTech Inc.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.17/H40

Topic: C.19. Drug Discovery and Development

Support: Supported by the Alzheimer's Association to GMP and PBR.

Title: Clinical development of a bioactive dietary polyphenol preparation for treating patients with mild cognitive impairment and prediabetes

Authors: *L. DUBNER[1], L. HO[1], J. WANG[1,2], P. B. ROSENBERG[3], G. M. PASINETTI[1,2]; [1]Dept. of Neurol., Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Geriatric Res. Educ. and Clin. Ctr., James J. Peters Veterans Affairs Med. Ctr., Bronx, NY; [3]Divisions of Geriatric Psychiatry and Neuropsychiatry, Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Mild cognitive impairment (MCI) is an important target population for prevention of Alzheimer's disease (AD). Diabetes is a major cause of vascular problems and is associated with cognitive decline in older people, either through vascular mechanisms or by directly affecting the AD development process. Prediabetes is defined as the state of having an elevated fasting blood sugar level, increasing the risk of developing diabetes in the near future. Moreover, there is evidence that people with MCI and prediabetes are at particularly high risk of progressing to AD. There are currently no FDA-approved treatments for comorbid MCI and prediabetes. In addition, there is evidence that drugs controlling blood sugar may be detrimental to cognition. Thus, there is a great need for new interventions to prevent people with MCI and prediabetes from progressing to AD. We have strong preclinical preliminary data on the potential efficacy of a combination of three nutraceuticals (a Bioactive Dietary Polyphenol Preparation [BDPP]) in targeting amyloid load, synaptic plasticity, and cognition in mouse models of AD and metabolic syndrome. Based on this and of the safety profile of orally administered BDPP, BDPP has great potential for secondary prevention of cognitive/functional decline in comorbid MCI with metabolic risk factors. We have evidence for excellent penetrance of orally administered BDPP in the central nervous system (CNS) of mice, but further evidence on BDPP CNS penetrance, safety, and tolerability in humans is needed in order to assess its potential as a therapeutic agent. Thus, we are currently conducting a 4-month, phase Ib, ascending-dose, randomized, placebo-

controlled, double-blind study of oral BDPP administration in 48 participants with MCI and prediabetes. Primary outcome measures are cerebrospinal fluid (CSF) penetrance and safety, with secondary outcome measures of change in cognition and in CSF amyloid and tau content. The results of our pilot studies may support a single-dose, proof-of-concept trial of BDPP in participants with MCI and prediabetes, conceivably leading to the development of BDPP as a secondary prevention in this important population. We will present evidence supporting the safety of BDPP, bioavailability of BDPP polyphenols in the brain, and the mechanism by which BDPP polyphenols may modulate multiple inter-relating AD pathogenic mechanisms. Lastly, we will present an update of our ongoing BDPP clinical trial.

Disclosures: L. Dubner: None. **L. Ho:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LH is among the inventors of a patent using grape seed extract (GSE) in neurodegenerative diseases (Patent number 8747924; Methods for preventing and treating neurodegenerative diseases). **J. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); JW is among the inventors of a patent using grape seed extract (GSE) in neurodegenerative diseases (Patent number 8747924; Methods for preventing and treating neurodegenerative diseases).. **P.B. Rosenberg:** None. **G.M. Pasinetti:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GMP is among the inventors of a patent using grape seed extract (GSE) in neurodegenerative diseases (Patent number 8747924; Methods for preventing and treating neurodegenerative diseases)..

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.18/H41

Topic: C.19. Drug Discovery and Development

Support: TWU Department of Biology

Research Enhancement Program

Closing the GAPs Prgoram

Undergraduate Microgrant programs

Title: Active cytosolic Rac1 and nuclear RhoA: Effects on neurite morphology

Authors: *J. M. REDDY, N. G. R. RAUT, D. L. HYNDS;
Biol., Texas Woman's Univ., Denton, TX

Abstract: Rho family guanine nucleotide triphosphatases (GTPases) are molecular switches that play a fundamental role in axon growth and guidance by directing growth cone cytoskeletal changes through interaction with downstream effectors. Small GTPases are considered active when bound to guanosine triphosphate (GTP), a process promoted by plasma membrane-associated guanine exchange factors (GEFs), and inactive when bound to guanosine diphosphate (GDP). RhoA, a member of the Rho family GTPases, participates in the formation of focal adhesions, retraction of neuron processes in response to injury or insult and growth cone collapse. Rac1, thought to be a competitor of RhoA, is responsible for cell proliferation, cell cycle entry, and extension of neuronal processes. The interplay between Rac1 and RhoA dynamics is not well elucidated, though they are thought to be direct competitors with opposing functions. Both require prenylation for membrane localization, though active forms of both have been found in other cellular compartments (GTP bound Rac1 in the cytosol and GTP RhoA primarily in the cytosol and nucleus). We constructed non-prenylatable a Rac1 and RhoA to test how inhibiting prenylation affects morphology and the location of active RhoA and Rac1. Non-prenylatable Rac1 induced cell clustering in B35 neuroblastomas, hallmarks of degeneration, while non-prenylatable RhoA increased neurite outgrowth. Non-prenylatable Rac1 was found active in the cytosol in both B35 neuroblastomas and rat cortical neurons while non-prenylatable RhoA was localized to the membrane, cytosol and nucleus. In fact, RhoA retained the ability to be made active independent of membrane targeting by prenylation after treatment with lovastatin. We have found transfection of these constructs in rat cortical neurons increase neurite outgrowth (for non-prenylatable RhoA and wildtype Rac1), neurite branching (for RhoA) and neurite formation (for non-prenylatable Rac1). With emerging evidence of differential activation of these Rho GTPases based on their subcellular localization, elucidating the signaling cascades of the active GTPases may identify novel targets to facilitate axon regeneration in traumatic or degenerative neurological conditions.

Disclosures: J.M. Reddy: None. **N.G.R. Raut:** None. **D.L. Hynds:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

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Program#/Poster#: 54.19/H42

Topic: C.19. Drug Discovery and Development

Support: FRAXA Research Foundation

Grants to Enhance and Advance Research-UH

Title: Regulation of rac1-gtp by statin therapy in a genetically modified mouse model of autism spectrum disorders

Authors: Q. LING, *M. V. TEJADA-SIMON;
Dept Pharmacol & Pharma Sci., Univ. Houston, Houston, TX

Abstract: Rac1, one of the small Rho GTPases, is a critical mediator of dendritic spine development and long-term plasticity in the brain. Significant up-regulation of Rac1 has been documented in fragile X mental retardation 1 (fmr1) knockout mice, an animal model for autism spectrum disorder (ASD), suggesting that loss of Fragile X Mental Retardation Protein (FMRP) affects Rac1 activity. Interestingly, abnormal phenotypes described for this rodent model (e.g., cognitive deficits, risk of audiogenic seizures, altered long-term plasticity) are rescued upon normalizing levels of Rac1 by using specific pharmacological inhibitors. Since those Rac1 inhibitors have not been clinically developed yet, it is imperative to find an alternative way to benefit ASD patients. To be functional, Rac1 needs to undergo a post-translational modification in which an isoprenoid group is added to the protein, facilitating its subcellular localization and association with specific membranes. These prenylated proteins are uniquely formed through the mevalonate/cholesterol pathway, an important metabolic pathway for numerous cell functions. Based on our previous research, we hypothesized that restricting the availability of prenylated proteins by inhibiting the central mevalonate pathway with statins (3-hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] competitive inhibitors) might also control Rac1 activation and function in the fmr1- knockout mice, similar to our previous results with the specific Rac1 inhibitors. To test this hypothesis we first investigated whether statin therapy reduces Rac1-GTP in fmr1 knockout mice. We chose Rosuvastatin as a test drug because of its higher bioavailability and higher half-life time compared to other statins. We incubated hippocampal slices derived from fmr1- knockout mice (FVB129 background) with different concentrations of Rosuvastatin (0-20μM) for 2 hours. Levels of active Rac1 (Rac1-GTP bound) by G-LISA as well as total Rac1 by Western Blots were determined. Our preliminary results suggested that, while statin treatment showed a slight trend to enhance total Rac1 content, activated Rac1 (Rac1-GTP) was still significantly decreased, suggesting that statin treatment might indeed be effective in reducing the prenylation of this small GTPase, hindering its membrane translocation and full activation. These findings indicated that regulation of isoprenoids might be useful to regulate overactivation of Rac1, and suggested that specific inhibition of protein prenylation could be a potential therapeutic strategy to rescue cognitive and plasticity deficits associated with neurodevelopmental diseases.

Disclosures: Q. Ling: None. **M.V. Tejada-Simon:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.20/H43

Topic: C.19. Drug Discovery and Development

Support: NIH Phase II SBIR AG039129

Title: PTI-51-CH3 (TauPro™) and PTI-80 are novel small molecule tau aggregation inhibitors and pre-clinical candidates for the treatment of tauopathies

Authors: K. L. HANSON, J. CAM, J. CUMMINGS, L. A. ESPOSITO, T. LAKE, T. M. CHONG, A. D. SNOW, Q. HU;
ProteoTech, Inc., Kirkland, WA

Abstract: Accumulation of intracellular neurofibrillary tangles (NFTs) composed of aggregated tau protein is a key pathological hallmark of Alzheimer's disease and other tauopathies such as Progressive Supranuclear Palsy (PSP). Lead compounds PTI-51-CH3 (TauPro™) and PTI-80 were identified as highly potent tau aggregation inhibitors through a screen of ProteoTech's proprietary small molecule library. PTI-51-CH3 and PTI-80 both prevent tau aggregation at substoichiometric proportions in a dose-dependent manner as measured by Thioflavin S fluorometry, circular dichroism spectroscopy and electron microscopy. Drugability studies demonstrate a favorable profile, with no potent reversible inhibition of major cytochrome P450 enzymes and reasonable stability in plasma and brain homogenates. Compound formulation studies in support of pharmacokinetic studies demonstrate that both compounds are readily stable (>95%) in formulations for at least several weeks. Importantly, PK studies in mice reveal that PTI-51-CH3 and PTI-80 provide good plasma, brain and CSF exposure after subcutaneous injection. For example, brain levels of both compounds at the Cmax significantly exceed estimated free tau in brain cells, suggesting that PTI-51-CH3 and PTI-80 are present in the brain at levels sufficient to modulate tau aggregation. PTI-51-CH3 and PTI-80 are currently being tested for *in vivo* efficacy in a transgenic mouse model that expresses a human tau isoform with a FTDP-17 P301S mutation that is commonly used as a tauopathy animal model. Initial results from tau transgenic mice treated with PTI-51-CH3 and PTI-80 (versus vehicle) will be presented. Our studies establish PTI-51-CH3 (TauPro™) and PTI-80 as top pre-clinical candidates for development as tau aggregation inhibitors for the treatment of Alzheimer's disease, Progressive Supranuclear Palsy and other tauopathies.

Disclosures: **K.L. Hanson:** A. Employment/Salary (full or part-time):; ProteoTech. **J. Cam:** A. Employment/Salary (full or part-time):; ProteoTech. **J. Cummings:** A. Employment/Salary (full or part-time):; ProteoTech. **L.A. Esposito:** A. Employment/Salary (full or part-time):; ProteoTech. **T. Lake:** A. Employment/Salary (full or part-time):; ProteoTech. **T.M. Chong:** A. Employment/Salary (full or part-time):; ProteoTech. **A.D. Snow:** A. Employment/Salary (full or part-time):; ProteoTech. **Q. Hu:** A. Employment/Salary (full or part-time):; ProteoTech.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.21/H44

Topic: C.19. Drug Discovery and Development

Support: Funding was provided by NIH-NCCAM grant P01AT004511-01 as part of the Mount Sinai School of Medicine Center of Excellence in Complementary and Alternative Medicine for Alzheimer's Disease (to GM Pasinetti)

Title: Intestinal microbiota-derived phenol acids are capable of accumulating in the brain and interfere with β-amyloid oligomerization

Authors: *L. HO[1], J. FAITH[2], G. M. PASINETTI[3,4];
[1]Dept Neurol., [2]Dept. of Genet. and Genomic Sci., [3]Dept. of Neurol., The Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Geriatric Res. and Clin. Ctr., James J. Peters Veterans Affairs Med. Ctr., Bronx, NY

Abstract: Grape seed polyphenol extract (GSE) is receiving increasing attention for its potential preventative and therapeutic roles in Alzheimer's disease (AD) and other age-related neurodegenerative disorders. The intestinal microbiota is known to actively convert many dietary polyphenols, including GSE, to phenolic acids. There is limited information on the bioavailability and bioactivity of GSE-derived phenolic acid in the brain. Thus, we orally administered GSE to rats and investigated the bioavailability, in both plasma and in the brain, of 12 phenolic acids that are known to be generated by microbiota metabolism of polyphenols. We found GSE treatment significantly increased the content of two phenolic acids in the brain: 3-hydroxybenzoic acid (3-HBA) and 3-(3´-hydroxyphenyl) propionic acid (3-HPP), resulting in the brain accumulations of the two phenolic acids at μM concentrations. In pilot studies, we explored the feasibility of using germ-free mice and conventionalized mice where germ-free mice were colonized with the intestinal contents of a conventional mouse to study conversion of GSE into brain-available phenolic acids. We found ~8-fold lower plasma contents of 3-HPP in

germ-free versus conventionalized mice. Ongoing studies are analyzing plasma contents of 3-HBA, as well as brain contents of 3-HBA and 3-HPP in germ-free versus conventionalized mice. Lastly, we investigated effects of 3-HBA and 3-HPP on interfering with oligomerization of β-amyloid (Aβ) peptides that plays key roles in AD pathogenesis. Using multiple *in vitro* complementary assays we demonstrated that both 3-HBA and 3-HPP potently interfere with the assembly of Aβ peptides into neurotoxic Aβ aggregates. Our observation suggests important contribution of the intestinal microbiota to the protective activities of GSE (as well as other polyphenol preparations) in AD. Outcomes from our studies support future preclinical and clinical investigations exploring the potential contributions of the intestinal microbiota in protecting against the onset/progression of AD and other neurodegenerative conditions. Moreover, our evidence validates the utility of germ-free and gnotobiotic animals as a platform to study the effects of microbiota on phenolic acid bioavailability.

Disclosures: **L. Ho:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LH is among the inventors of a patent using grape seed extract (GSE) in neurodegenerative diseases (Patent number 8747924; Methods for preventing and treating neurodegenerative diseases).. **J. Faith:** None. **G.M. Pasinetti:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GMP is among the inventors of a patent using grape seed extract (GSE) in neurodegenerative diseases (Patent number 8747924; Methods for preventing and treating neurodegenerative diseases)..

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.22/H45

Topic: C.19. Drug Discovery and Development

Support: TWU Dept. Biology

TWU Research Enhancement Program

TWU Undergraduate Microgrant Program

TWU Closing the Gaps program

Title: Implications of activated Rho GTPases in different subcellular locations

Authors: *D. L. HYNDS, N. G. R. RAUT, R. CHABAYTA, K. RHODEN, F. SAMUEL, J. REDDY;
Texas Woman's Univ., Denton, TX

Abstract: Precise regulation of activity in the Rho family of guanosine triphosphatases (GTPases) is necessary for proper development and functioning of the nervous system, and alterations in Rho GTPase signaling are implicated in a variety of neurodegenerative and neurotraumatic disorders. Rho GTPase activation is regulated by both membrane localization, through the addition of geranylgeranyl groups, and through interactions with activation guanosine exchange factors (GEFs) and inactivation guanosine dissociation inhibitors GDIs) and GTPase activating proteins (GAPs). It is commonly assumed that Rho GTPases must be translocated to the plasma membrane to interact with GEFs to become activated. However, investigations into the cellular spatial and temporal patterns of Rho GTPase activation have indicated that a portion of each of the prototypical GTPases (RhoA, Rac1 and Cdc42) are active in the cytosol of neurons or neuron-like cells. To further define the contribution of this pool of GTPases to mediating neurite outgrowth, we have generated and expressed non-prenylatable Rho GTPase mutants in neuron-like cell lines and primary cultures of cortical neurons. As expected, expression of non-prenylatable RhoA or wild-type Rac1 increased neurite outgrowth, with expression of non-prenylatable Rac1 and wild-type RhoA decreasing outgrowth. Interestingly, expressing non-prenylatable Rho GTPases also affected downstream signaling from well-known cell differentiation pathways (e.g. ERK 1/2 and JNK) and actin binding proteins (e.g. Arp2/3, WAVE and cofilin). Since prenylation, and subsequent membrane localization, of Rho GTPases is suggested to be altered in ageing and neurodegeneration, understanding the differential effects of subcellular Rho GTPase localization on signaling pathways is important for deciphering the pathology of and designing therapies for a host of neurodevelopmental, neurodegenerative and neurotraumatic disorders.

Disclosures: D.L. Hynds: None. **N.G.R. Raut:** None. **R. Chabayta:** None. **K. Rhoden:** None. **F. Samuel:** None. **J. Reddy:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.23/H46

Topic: C.19. Drug Discovery and Development

Support: Melendy/Peters Research Scholarship, College of Pharmacy, University of Minnesota

Faculty Research Development Program, Academic Health Center, University of Minnesota

Title: Upregulation of protein farnesylation in Alzheimer's disease

Authors: A. JEONG[1], D. CAO[1], S. CHENG[1], M. DISTEFANO[2], D. BENNETT[3], *L. LI[1]; [1]Exptl. and Clin. Pharmacol., [2]Chem., Univ. of Minnesota, Minneapolis, MN; [3]Neurolog. Sci., Rush Univ., Chicago, IL

Abstract: The pathogenesis of Alzheimer's disease (AD), particularly the sporadic form of AD, is not fully understood. Emerging evidence indicates that isoprenoids and prenylated proteins may play an important role in the pathogenesis of AD. Prenylation reactions are catalyzed by prenyltransferases, including farnesyl transferase (FT) and geranylgeranyl transferase (GGT) type-1 and -2. FT and GGT attach isoprenoids to proteins, which facilitates anchoring of proteins in cell membranes and mediates protein-protein interactions. A variety of important intracellular proteins, including almost all small GTPases such as members of Ras, Rho, and Rab subfamilies, undergo prenylation for proper location and function. Small GTPases are involved in regulating a wide range of cellular processes and functions. The levels of isoprenoids have been shown to be elevated in brains of Alzheimer's patients. Recently, we have demonstrated that protein farnesylation and geranylgeranylation pathways play distinct neurophysiological roles and that reducing farnesylation, but not geranylgeranylation, rescues cognitive function as well as attenuates AD-like pathology in the APP/PS1 transgenic mouse model of AD. However, the prenylation state of proteins in AD has not been investigated. This study is undertaken to determine the level of prenylated proteins in brain tissues from both AD model mice and human subjects with a spectrum of cognition from normal, mild cognitive impairment (MCI), to AD dementia. The levels of representative FT and GGT substrate small GTPases, including RhoA, H-Ras, and Rab7, in total brain homogenate and subcellular (membrane-bound and cytosolic) fractions were measured. The levels of relevant downstream signaling molecules including ERK were also quantified. Our preliminary results showed that the level of membrane-bound (farnesylated) H-Ras was significantly increased in the brain of APP/PS1 mice, whereas there was no change in the level of geranylgeranylated RhoA and Rab7. Consistent with the results in AD mice, there was a significant increase in membrane-bound H-Ras levels in MCI and AD brains compared to those in normal cognition brains. Also, the level of membrane-bound H-Ras was significantly correlated with the tangle burden in the brain. In addition, there was an increase in the activated form of ERK (p-ERK) in MCI brains. Taken together, these findings suggest that farnesylated small GTPases and downstream signaling pathways are upregulated in the early stage of AD and that farnesyltransferase could be a potential target for therapeutic intervention.

Disclosures: A. Jeong: None. **D. Cao:** None. **S. Cheng:** None. **M. Distefano:** None. **D. Bennett:** None. **L. Li:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.24/H47

Topic: C.19. Drug Discovery and Development

Support: College of Pharmacy - University of Minnesota

Academic Health Center - University of Minnesota

NIH/NIA training grant: Functional Proteomics of Aging T32 AG029796

Title: Deficiency of geranylgeranyltransferase-1 reduces spine density and synaptic plasticity

Authors: *D. A. HOTTMAN[1], S. CHENG[1], L. YUAN[2], M. BERGO[4], W. G. WOOD[3], L. LI[1]; [1]Exptl. and Clin. Pharmacol., [2]Neurosci., [3]Pharmacol., Univ. of Minnesota, Minneapolis, MN; [4]Inst. of Med., Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Isoprenoids and prenylated proteins are involved in regulating a variety of cellular functions including neurite growth and synaptic plasticity and are implicated in the pathogenesis of a number of diseases including Alzheimer disease (AD). Recently, we have demonstrated that the two protein prenyltransferases, farnesyltransferase (FT) and geranylgeranyltransferase-1 (GGT), differentially affect AD-related neuropathology and cognitive function in a transgenic mouse model. Heterozygous deletion of FT reduced amyloid-β (Aβ) deposition and neuroinflammation and rescued spatial learning and memory function in AD mice. In contrast, heterozygous deletion of GGT reduced Aβ levels and neuroinflammation but failed to rescue learning or memory function in AD mice. The current study investigated mechanisms that may account for the lack of cognitive benefits despite attenuated neuropathology in GGT haplodeficient mice. Preliminary data showed that the magnitude of long-term potentiation (LTP) was markedly suppressed in hippocampal slices from GGT-haplodeficient mice compared with wild-type or FT-haplodeficient mice. Consistent with the synaptic dysfunction, there was a significant decrease of postsynaptic protein PSD95 in the synaptosomal preparation from GGT-haplodeficient mice. To further study the neuronal effects of GGT deficiency, we have generated conditional neuron-specific GGT knock-out (GGTnKO) mice. In these mice, the level of GGT was reduced in the forebrain in an age-dependent manner. Preliminary studies showed that the dendritic spine density was significantly decreased in cortical neurons of GGTnKO mice compared to wild-type mice. Immunoblot analyses showed that there was a 4-fold reduction of membrane associated (prenylated) RhoA in GGTnKO mice, suggesting that insufficient prenylation of Rho family of small GTPases may underlie the negative effects of GGT deficiency. This also implies the critical role of GGT in maintaining spine structure and synaptic

plasticity. Our data are in line with recent findings that GGT is downregulated in the brain of aged mice, leading to a decrease of prenylated RhoA family of proteins, which may cause age-related weakening of synaptic plasticity. Experiments are underway to study the downstream signaling pathways leading to spine loss and synaptic dysfunction associated with GGT deficiency.

Disclosures: D.A. Hottman: None. **S. Cheng:** None. **L. Yuan:** None. **M. Bergo:** None. **W.G. Wood:** None. **L. Li:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.25/H48

Topic: C.19. Drug Discovery and Development

Support: Department of Veterans Affairs Career Development Award- KAL

NIH NINDS- EBS

Department of Veterans Affairs- EBS

Title: Statin-encapsulating nanoparticles as a potential therapeutic strategy for the management of inflammatory peripheral nerve disorders

Authors: *K. A. LANGERT[1], E. B. STUBBS, Jr[2];
[1]Res. Service, Hines VA Hosp., Hines, IL; [2]Res. Service, Edward Hines Jr. VA Hosp., Hines, IL

Abstract: Inflammatory peripheral neuropathies constitute one of the largest and least understood spectrums of neurologic disorders. Inclusive among these disorders is acute inflammatory demyelinating polyradiculopathy (AIDP), a debilitating autoimmune disease that affects the peripheral nervous system. Despite its overwhelming prevalence, treatment remains palliative and relies on the use of non-specific immune-modulating therapies. While the mechanisms that govern disease onset and progression are not completely understood, trafficking of autoreactive leukocytes across the blood-nerve barrier and into peripheral nerves is an early pathological hallmark. Our group has reported that therapeutic administration of statins safely attenuates the clinical severity of an animal model of AIDP (experimental autoimmune neuritis, EAN) by restricting leukocyte migration. Statins, in addition to inhibiting HMG-CoA reductase, inhibit the activation and signaling of monomeric GTPases. Using a novel cell line of the blood-nerve barrier, we demonstrated *in vitro* that leukocyte trafficking is dependent on active

monomeric GTPases. Our data suggest that Cdc42 and RalA, specific subfamilies of geranylgeranylated GTPases, promote release of chemotactic cytokines from peripheral nerve microvascular endothelial cells. Despite these advancements, the clinical application of systemically-administered statins for the management of inflammatory disorders remains controversial. Here, we examined the feasibility of a non-systemic drug-delivery system as an alternative means to locally administer statins for the management of EAN. Biodegradable lovastatin-encapsulating poly(lactic co-glycolic) acid (PLGA) nanoparticles were synthesized and characterized. The mean diameter of synthesized particles was 257.2 ± 7.0 nm as measured with dynamic light scattering and confirmed with scanning electron microscopy. Encapsulated lovastatin was quantified by HPLC. Drug loading ranged from 5.1 - 6.6% with an encapsulation efficiency of 81.6 - 91.5%. The use of biodegradable statin-encapsulating nanoparticles offers the advantage of a testable non-systemic, localized, delivery strategy by which to better manage difficult-to-treat inflammatory peripheral nerve disorders.

Disclosures: **K.A. Langert:** None. **E.B. Stubbs:** None.

Poster

054. Neurodegeneration Drug Discovery

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 54.26/I1

Topic: C.19. Drug Discovery and Development

Support: Department of Veterans Affairs (C7506M and I21RX001593 to CLP; C3638R, B3756-F & I21RX001553 to EBS)

Midwest Eye-Banks

Illinois Society for the Prevention of Blindness

Richard A. Perritt Charitable Foundation

Title: Geranylgeranylation regulates Rho GTPase mRNA expression and protein stability

Authors: *C. L. PERVAN[1,2], E. B. STUBBS, Jr[1,2];
[1]DVA, Edward Hines, Jr. VA Hosp., Hines, IL; [2]Ophthalmology, Loyola Univ. Chicago, Maywood, IL

Abstract: Post-translational addition of 15-carbon farnesyl and 20-carbon geranylgeranyl isoprenoids to conserved -CaaX motifs on small monomeric Rho GTPases precedes their target

membrane localization and subsequent activation (GTP-binding). Using cells isolated from the trabecular meshwork (TM), a specialized tissue within the eye that regulates intraocular pressure (IOP), our laboratory has expanded this field to include a novel role for isoprenoids in regulation of Rho GTPase mRNA and protein expression. Here, we demonstrate that inhibition of geranylgeranylation with the HMG-CoA reductase inhibitor lovastatin (10 μM, 24h) or a geranylgeranyl transferase inhibitor (GGTI-298; 10 μM, 24h), significantly enhances mRNA and protein content of RhoA and RhoB GTPases in both primary and transformed human TM cells. Quantifiable increases in RhoA expression occur as a result of enhanced protein stability. By comparison, inhibition of geranylgeranylation induces *de novo* mRNA and protein synthesis of RhoB, and increases stability of RhoB proteins. Accumulated Rho GTPases remain compartmentalized within the cytosol in an inactive (GDP-bound) state. In marked contrast, facilitating geranylgeranylation of accumulated, inactive Rho GTPases markedly enhances their target membrane distribution, functional activation (GTP-binding), and rapid degradation by the 20*S* proteasome. Collectively, this study demonstrates that post-translational geranylgeranylation represents a novel mechanism by which newly-synthesized Rho GTPases are translocated to target membranes, activated, and subsequently degraded. We hypothesize that a geranylgeranylation-dependent mechanism governing Rho GTPase expression and turnover represents a novel means by which intracellular Rho GTPase signaling is regulated.

Disclosures: **C.L. Pervan:** None. **E.B. Stubbs:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.01/I2

Topic: C.14. Gene Therapy

Support: CNPq

CAPES

Faperj

Title: Evaluation of olfactory ensheathing glia gene reprogramming by recombinant adeno-associated viral vector type 2 *in vitro* and *in vivo*

Authors: *L. A. CARVALHO[1], L. C. VITORINO[2], H. PETRS-SILVA[2], S. ALLODI[2];
[1]Univ. Federal do Rio de Janeiro, Rio De Janeiro, Brazil; [2]Inst. de Biofísica Carlos Chagas Filho, Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: The olfactory ensheathing glia (OEG) is a special type of glial cells that ensheath the axons of olfactory receptors of the olfactory mucosa. Axonal bundles of receptor axons extend toward the cribiform plate and enter the brain via the olfactory bulb, providing a direct route for pathogens to access the brain from the periphery. OEG has been used in cell therapy studies after spinal cord lesions and/or neurodegenerative diseases, with little success regarding the functional recovery of the central nervous system (CNS). Therefore, we intend to use a new approach: gene reprogramming the OEG into a pump of neurotrophic factors *in situ* by recombinant adeno-associated viral vector type-2 (rAAV-2 WT) through intranasal delivery and evaluate if the self-complementary AAV-2 mutant Y444F (scAAV-2 MUT Y444F), which contains single-point mutations of surface exposed tyrosine residues in the VP3 capsids, could significantly increase gene transduction efficiency *in vitro* and *in vivo*. Our preliminary results *in vitro* indicate that both rAAV-2 WT and scAAV-2 MUT Y444F containing the reporter green fluorescent protein (GFP), supplied at 1x10E8vg/µl in culture medium (DMEM/ F12 supplemented with 10% fetal bovine serum and antibiotics) after 7 days from infection was the best condition compared with other times (10 and 14 days) and with other dilutions (1x10E6vg/µl and 1x10E7vg/µl). To assess this issue *in vivo*, we conducted the technique of dot blot using brain samples dissected into three regions: 1) a region encompassing the olfactory bulb and the most cephalic portion of the cortex; 2) a region encompassing the mesencephalon and the hippocampus; and 3) the cerebellum and the medulla. We observed that, after intranasal instillation of rAAV-2 WT-GFP (1x10E9vg/µl, diluted in 10µl sterile NaCl 0,9%), the three cerebral regions were strongly labeled by the GFP antibody, showing that the gene transduction was successful. Our results indicate that the OEG can be used as pump of neurotrophic factors to CNS *in situ* by gene reprogramming with rAAV-2, delivered directly through the olfactory pathway, providing a new therapeutical approach for neurodegenerative diseases and CNS injuries.

Disclosures: L.A. Carvalho: None. **L.C. Vitorino:** None. **H. Petrs-Silva:** None. **S. Allodi:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.02/I3

Topic: C.14. Gene Therapy

Support: Association Française contre les Myopathies - AFM14814

LABEX LIFESENSES - ANR-10-LABX-65

Title: Trans-splicing gene therapy for dominant diseases: evidence of *in vivo* efficiency in a mouse model of retinitis pigmentosa induced by mutation of the rhodopsin gene

Authors: *A. .-P. BEMELMANS[1], A. BERGER[2], S. MAIRE[1], C. JOSÉPHINE[1], M. DESROSIERS[2], M.-C. GAILLARD[1], P. HANTRAYE[1], J.-A. SAHEL[2];
[1]CEA, Fontenay Aux Roses, France; [2]Inst. de la Vision, Univ. Paris VI, Paris, France

Abstract: Point mutations in the rhodopsin gene (RHO) are the most frequent cause of retinitis pigmentosa (RP), a group of inherited retinal dystrophy leading to blindness. Most of these mutations result in a gain of function or a dominant negative effect deleterious to the photoreceptors, thus leading to a dominant mode of transmission. In addition, it has been shown that variations in RHO expression level are also deleterious to the retina. A gene therapy strategy for RHO mutations should lead to both the suppression of expression of the mutant protein and restoration of a physiological level of the normal protein. Spliceosome-mediated RNA trans-splicing technology (SMaRT) should in theory respect these constraints by repairing mutations in the enbdogenous RHO pre-mRNA. This technology consists in introducing, by gene transfer, an exogenous RNA - called PTM, for pre-trans-splicing RNA molecule, capable of binding to the endogenous pre-mRNA and promoting a trans-splicing reaction, thus leading to replacement of the mutant portion of RHO pre-mRNA. We designed fourteen RHO-PTM, able to repair any mutations in exons 2, 3, 4 and 5, which differ only by their binding sequence to intron 1 of RHO pre-mRNA. To determine the effectiveness of each PTM, we co-transiently transfected HEK293T cells with a plasmid encoding the PTM to be tested and a RHO-expressing plasmid. The best candidate led to a trans-splicing efficiency of about 25% at the mRNA level. We increased this efficiency to 40% by refining the PTM sequence, consisting in the reintroduction of an endogenous RHO intron in the cDNA portion of the PTM. We then tested the PTM at the protein level in a cellular model expressing RHO stably. We precisely quantified the phenotype of cells expressing the mutant gene by a flow imaging method (ImageStreamX), and showed that trans-splicing allows to partially restore this phenotype. We then built an AAV vector encoding our best PTM to assess its *in vivo* activity after subretinal injection in a mouse model expressing the mutant RHOP347S. By co-injection with an AAV-GFP and micro-dissection of the transduced part of the retina, we have shown that trans-splicing reached 30% at the RNA level, while it was not detectable in non-transduced part of the retinaor in animals that received a control PTM. This study shows that trans-splicing may provide a therapeutic benefit in the case of dominant mutations of the rhodopsin gene.

Disclosures: A.-. Bemelmans: None. **A. Berger:** None. **S. Maire:** None. **C. Joséphine:** None. **M. Desrosiers:** None. **M. Gaillard:** None. **P. Hantraye:** None. **J. Sahel:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.03/I4

Topic: C.14. Gene Therapy

Support: Craig H. Neilsen Foundation

Merit Review Funding from the Department of Veterans Affairs

Department of Defense

New York State Spinal Cord Injury Research Board

Title: Non-invasive intrathecal AAV-10 vector-mediated administration of Neurotrophin-3 (AAV10-NT3-gfp) at chronic stage of contusion spinal cord injury (SCI) in rats

Authors: *V. L. ARVANIAN[1,2], H. PETROSYAN[1], V. ALESSI[1], J. M. LEVINE[2];
[1]VA Med. Ctr., Northport, NY; [2]Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Insufficient neurotrophin (NT-3) support is one of the major factors restricting synaptic transmission and plasticity in the damaged spinal cord. From our previous studies: although administration of NT-3 alone immediately after SCI has not shown significant improvements, NT-3 is an essential component of combination treatment. In an attempt to design an approach for prolonged delivery of NT-3, we have successfully created the new AAV10-NT3-gfp construct. Our previous experiments revealed an excellent transduction of neurons and glial cells in damaged spinal cord, after two weeks following thoracic contusion and *intraspinal* injections of AAV10-NT3-gfp, immediately after contusion injury. In this study, we sought to examine a more clinically relevant approach for AAV vector administration, i.e. *intrathecal* administration of AAV vector at chronic stage of injury. Adult rats received moderate contusion (150 kdyn) injury at thoracic T10 level using an IH impactor device. Locomotor performance was evaluated pre-injury, 2 days after SCI and then weekly for 6 weeks (automated Catwalk gait; Irregular Grid and Narrowing Beam tests). After 6 weeks post-injury, when the scores of all behavioral tests and gait analyses plateaued, rats were re-anesthetized and AAV10-NT3-gfp was administered intrathecally, using a 32 Gage Teflon catheter that was inserted through a small hole in the dura (made by needle) one segment caudal to injury, with the tip of the catheter positioned just caudal to the injury. Consistent with the results of previous experiments that examined acute effects of NT-3 alone, we found that delayed administration of AAV10-NT3-gfp alone has not induced improvements of locomotor function following chronic contusion SCI.

After completion of behavioral testing, spinal horizontal sections were prepared and unamplified GFP signal is under examination. So far, we have observed the GFP signal in axons and glial cells close to injury epicenter; although this signal was lower as compared to intraspinal injections. Quantitative analyses of the transduction efficacy of neurons and glia are still on-going. Although these experiments were important to examine effects of AAV10-NT3-gfp alone and to ensure transduction efficacy following intrathecal administration of AAV10-based vector at the chronic stage of SCI, further experiments will be conducted to examine effects of intrathecal administration of AAV10-NT3 combined with other treatments, i.e. AAV10-NG2-antibody, following chronic SCI.

Disclosures: V.L. Arvanian: None. **H. Petrosyan:** None. **V. Alessi:** None. **J.M. Levine:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.04/I5

Topic: C.14. Gene Therapy

Support: DOD Grant W81XVVH-12-1-0051

Title: Evaluation of three partial sequences of human TH promoter to specifically target dopaminergic neurons in a context of gene therapy for Parkinson's disease

Authors: *A.-S. ROLLAND, T. KAREVA, O. YARYGINA, N. KHOLODILOV, R. E. BURKE;
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Abstract: We have observed that increased Akt/mTor signaling in dopamine neurons of the substantia nigra (SN), achieved by transduction with AAV myristoylated-Akt (Myr-Akt) or AAV hRheb (S16H), two constitutively active mutants, induces axon re-growth weeks after their destruction by neurotoxin (Kim et al, Ann Neurol 2011; Mol Ther, 2012). However, both of these genes are potent oncogenes, so strategies must be devised to avoid their potential to transform cells before they can be considered safe for clinical use. One strategy would be to restrict gene expression to the desired neuron target and avoid expression in glia which have neoplastic potential. It may be possible to limit expression specifically to dopamine (DA) neurons by use of the TH promoter. Previous work has shown that transgene expression in living animals and cell lines can be achieved with a number of partial sequences of varying sizes of human TH (hTH) promoter: 11kb (Kaneda et al, 1991), 5kb (Sasaoka et al, 1992), 3.3kb (Kim et

al, 2003), a combination of 5' and 3' flanking region (Gardaneh et al, 2000), 522bp (Coker et al, 1988; Steffers et al, 2004). We therefore undertook to find the optimal human TH promoter among those that can be accommodated by the capacity limits of an AAV vector (i.e. ≤4.7kb). In order to assess expression in DA neurons and evaluate efficiency and specificity we designed constructs in which each of three hTH promoter sequences drove expression of a photostable red fluorescent protein TagRFP-T: hTHp-522-TagRFP-T, hTHp-5,3-TagRFP-T and hTHp-3.3-TagRFP-T. 6 weeks after injection of AAV in SNpc, we observed expression of TagRFP-T in TH-positive neurons and in both subpopulations of dopaminergic neuron, the AHD2-positive neurons and calbindin-positive neurons for all three constructs. Moreover, no expression was observed following injection of striatum or cortex which contain few, if any, DA neurons. For all three constructs we never observed expression in glial cells identified by GFAP fluorescent staining. To assess the level of expression mediated by these promoters, the number of TagRFP-T-positive cells in the entire SN was determined: hTHp-5,3-TagRFP-T (517±81.8) > hTHp-3.3-TagRFP-T (328.8±50.2) > hTHp-522-TagRFP-T (251.5±30.7). The number of TagRFP-T-positive neurons in the SN that are Nissl-positive but also TH/Nissl-positive was determined to evaluate specificity and efficiency. We found that hTHp-522-TagRFP-T had the highest sensitivity (76%) and specificity (20%). We conclude that the human 522bp TH promoter in an AAV vector can be useful to mediate expression of genes with therapeutic potential such as Akt or hRheb (S16H).

Disclosures: **A. Rolland:** None. **T. Kareva:** None. **O. Yarygina:** None. **N. Kholodilov:** None. **R.E. Burke:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.05/I6

Topic: C.14. Gene Therapy

Support: Royal Society of NZ Marsden Fund

Title: Adeno-associated viral vector serotype 5 (AAV5) mediates efficient neuronal and astrocytic therapeutic transgene expression in the substantia nigra pars compacta (SNpc)

Authors: *J. MUDANNAYAKE, D. FONG, A. MURAVLEV, D. YOUNG;
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Abstract: Given that reactive astrocytes contribute to pathological processes in neurodegeneration, astrocytes are an equally appealing and important target for CNS gene therapy as neurons. The aim of this study was to achieve dual targeting of therapeutic transgene expression to neurons and astrocytes in the SNpc, leading to neuroprotection in a rat model of Parkinson's disease (PD). We firstly compared AAV serotypes 5 and 9 cell transduction profiles in the SNpc. AAV vectors expressing destabilised yellow fluorescent protein (dYFP) regulated by an astrocytic glial fibrillary acidic protein (GFAP) promoter (AAV-GFAP-dYFP) were packaged, and injected unilaterally into the SNpc of adult male Sprague-Dawley rats (n=3 per vector). At 3 weeks, immunohistochemical (IHC) analysis of transgene expression confirmed a superior targeting of both nigral neurons and astrocytes by AAV5. We then generated an AAV5 vector expressing hemagglutinin-tagged nuclear receptor related 1 (AAV5-GFAP-HA-NURR1), a transcription factor that maintain dopaminergic neurons (DA) and regulate inflammatory mediators in astrocytes. To determine neuroprotective potential of astrocytic and neuronal HA-NURR1 expression, AAV5 vectors expressing dYFP, an empty control or HA-NURR1 were injected into the SNpc (n = 4 per control vector; 8 per therapeutic vector). Degeneration of DA neurons and motor deficits were induced by injecting 6-OHDA (20μg) into the ipsilateral striatum 3 weeks post vector. The cylinder test at 4 and 8 weeks post toxin infusion revealed that, while control groups retained a motor bias at 8 weeks (-27.29% and -26.48% contralateral forepaw use relative to baseline in empty and dYFP vector groups respectively), HA-NURR1 animals exhibited a recovery in motor deficits (0.08%). At 8 weeks, stereological quantification of tyrosine hydroxylase (TH)-positive DA neurons indicated that compared to empty (38.9% surviving neurons relative to contralateral SNpc) and dYFP (43.8%) vector groups, a greater neuronal survival was evident in the HA-NURR1 (53.7%) treated SNpc. Lower immunoreactivity to astrocyte and microglia markers, GFAP and Iba1 respectively, in HA-NURR1 brains indicated reduced neuroinflammation. These results suggest that AAV5 vectors could be used to genetically manipulate nigral cells for therapeutic purposes. Supported by the Royal Society of NZ Marsden Fund

Disclosures: **J. Mudannayake:** None. **D. Fong:** None. **A. Muravlev:** None. **D. Young:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.06/I7

Topic: C.14. Gene Therapy

Title: Translating an optogenetic gene therapy approach for treatment of neuropathic pain in humans

Authors: C. TOWNE, J. AGUADO, A. ARGUELLO, C. B. DISCENZA, S. KHAN, T. GALFIN, S. GEHRKE, *S. P. BRAITHWAITE, M. G. KAPLITT;
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Abstract: Optogenetics has been established as a powerful tool to study the central and peripheral nervous systems with its potential for directly treating human disease tantalizingly on the horizon. Optogenetic inhibition of pain in mice has been shown to be effective and provides an attractive initial clinical application of this technology. We aim to translate this approach to treat neuropathic pain in humans. Firstly, we demonstrated that the inhibitory opsin, NpHR, delivered by intraneural injections of AAV6 could reduce pain (mechanical allodynia) in a clinically meaningful paradigm i.e. vector delivery after establishment of neuropathic pain in the chronic constriction injury (CCI) model. The inhibitory opsin, SwiChR (a chloride channel requiring less light than NpHR to pass currents) could also reduce pain in this system. We next tested a surgical approach more amenable to patients than nerve injection. Lumbar puncture is a routine clinical practice and AAV8 delivery by this method results in efficient gene delivery to sensory neurons in rodents, dogs and pigs. We found that intrathecal administration of AAV8 expressing SwiChR could decrease mechanical allodynia in the CCI mouse model. The magnitude of pain inhibition correlated with transduction levels and only 10% transduction of sensory neurons was required for pain relief. To further validate this approach we utilized a second rodent pain model with translational relevance. The mouse tibia fracture/cast immobilization model of Complex Regional Pain Syndrome (CRPS) presents extreme allodynia and recapitulates human disease effectively. We found that intrathecal delivery of AAV8 encoding SwiChR following development of the CRPS model resulted in light-mediated pain inhibition. Taken together, our results confirm optogenetic therapy for neuropathic pain as an attractive clinical application of this technology.

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Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.07/I8

Topic: C.14. Gene Therapy

Support: CHDI funding support

Title: Widespread gene delivery to the nonhuman primate brain for the treatment of Huntington's disease

Authors: *L. M. STANEK[1], P. HADACZEK[5], B. MASTIS[2], M. KELLY[3], C. O'RIORDAN[4], P. PIVIROTTO[5], J. BRINGAS[5], A. CIESIELSKA[5], W. SAN SEBASTIAN RAMIREZ[5], S. CHENG[1], K. BANKIEWICZ[5], L. SHIHABUDDIN[2];
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Abstract: Huntington disease (HD) is an autosomal dominant neurodegenerative disease caused by a CAG-trinucleotide repeat expansion in a coding exon of a single allele in the HTT locus. In HD, the resulting polyglutamine (polyQ) expansion confers a toxic gain-of-function to the mutant huntingtin protein (mHTT). Reduction of expression of mHTT using gene silencing by RNA interference (RNAi) may confer transformative disease modifying therapeutic approach for HD. Adeno associated vectors (AAV) provide an ideal delivery system for nucleic acid therapeutics and have the potential to allow for long lasting and continuous expression of these huntingtin lowering RNAi in the brain. Despite this promise, global delivery of AAV to the adult brain remains an elusive goal. Furthermore, the appropriate brain areas to target for achieving transformative therapeutic benefit in HD patients remain to be defined. Postmortem analyses of HD patient brains reveal extensive medium spiny neuronal loss in the striatum, in addition to loss of pyramidal neurons in the cerebral cortex and hippocampus. Recent studies in rodent models suggest that simultaneous targeting of striatum and cortex is more efficacious than targeting either individually. Thus, available evidence suggests that delivery of Htt-lowering therapeutics to both striatal and cortical regions may provide optimal therapeutic efficacy. The current study demonstrate for the first time the successful use of an AAV targeting strategy that leads to viral transduction in key brain areas considered to be important for HD pathology. The study compared the efficiency of transduction of AAV1 and AAV2 vectors in the rhesus monkey brain following intra-striatal injection. Both vectors encoded green fluorescent protein (GFP) under control of a hybrid CMV enhancer/chicken beta-actin promoter. One month following injection, brains were analyzed for distribution of GFP-positive cells. We found that the AAV1 vector provided extensive delivery to the majority of the primate striatum, and additionally transduced large numbers of cells within the cerebral cortex, thalamus, and hippocampus. In summary, the data suggest that intrastriatal delivery maybe sufficient to for the delivery of nucleic acid-based therapeutics to multiple areas of the human brain relevant in HD.

Disclosures: **L.M. Stanek:** A. Employment/Salary (full or part-time):; Genzyme, A Sanofi Company. **P. Hadaczek:** None. **B. Mastis:** A. Employment/Salary (full or part-time):; Genzyme, A Sanofi Company. **M. Kelly:** A. Employment/Salary (full or part-time):; Genzyme, A Sanofi

Company. **C. O'Riordan:** A. Employment/Salary (full or part-time):; Genzyme, A Sanofi Company. **P. Pivirotto:** None. **J. Bringas:** None. **A. Ciesielska:** None. **W. San Sebastian Ramirez:** None. **S. Cheng:** A. Employment/Salary (full or part-time):; Genzyme, A Sanofi Company. **K. Bankiewicz:** None. **L. Shihabuddin:** A. Employment/Salary (full or part-time):; Genzyme, A Sanofi Company.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.08/I9

Topic: C.14. Gene Therapy

Support: Florida Department of Health

Craig H. Neilsen Foundation 190926

Title: Evaluation of compound gene therapy in combination with physical exercise in the treatment of chronic SCI neuropathic pain

Authors: *S. JERGOVA[1], C. E. GORDON[1], S. GAJAVELLI[1], E. DUGAN[1], J. ZADINA[2], J. SAGEN[1];
[1]Miami Project, Univ. of Miami, Miller Sch. of Med., Miami, FL; [2]Dept Med., Tulane University, Southeast Louisiana Veterans Hlth. Care Syst., New Orleans, LA

Abstract: The insufficient pain relief provided by current pharmacotherapy for chronic neuropathic pain is a serious medical problem and development of new treatment strategies are of a high clinical interest. Mechanisms underlying chronic neuropathic pain include decreased spinal tonic inhibition due to the dysfunction of GABA signaling and increased excitatory signaling via NMDA receptors. Previous studies in our lab showed beneficial effects of the intraspinal transplantation of GABAergic neuronal precursor cells (NPCs) enhanced with the addition of serine histogranin (SHG), an NMDA receptor antagonist. In addition, our studies showed that combined injection of viral vectors engineered to produce SHG with another analgesic peptide vector, endomorphin-1 (EM-1) lead to enhanced analgesia. Other ongoing studies in our lab have shown positive effects of intensive treadmill locomotor training on alleviation of neuropathic pain behaviors in animals with SCI. The current study evaluated the combination of either recombinant cells or recombinant AAV vectors to deliver SHG and EM1 with treadmill training on pain behavior in rats after a clip compression SCI. Recombinant plasmids encoding single- and multi-SHG and EM-1 were engineered. Animals showing clear

signs of pain-related behavior at four weeks post-SCI were used for intraspinal injection of either recombinant NPCs or recombinant AAVs. Two weeks post injection animals treated with rAAVs showed significant attenuation of tactile sensitivity compared to controls and the antinociceptive effects were sustained up to 6 weeks post injection. More robust analgesic effects were observed in the group with 6SHG-EM1 construct compared to 1SHG-EM1. Animals treated with rNPCs did not display significant pain relief compared to the nonrecombinant NPCs group. However, some of the rNPC animals were included in the intensive treadmill training starting one week post grafting and these animals showed enhanced analgesic effects of the rNPCs. This study demonstrates that combination strategies taking advantage of multiple pain processing targets and including rehabilitation may provide enhanced benefits in reducing SCI neuropathic pain.

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Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.09/I10

Topic: C.19. Drug Discovery and Development

Title: *In vivo* 5-HT6 receptor occupancy- an assessment with radiolabeled and non-radiolabeled Lu AE60157 as a tracer in rats

Authors: J. THENTU, *S. M. IRAPPANAVAR, G. BHYRAPUNENI, R. ALETI, N. MUDDANA, A. SHINDE, R. BADANGE, R. NIROGI;
SUVEN LIFE SCIENCES LTD, HYDERABAD, India

Abstract: Selective 5-HT6 ligand, Lu AE60157 has been used as a tracer in the determination of *in vivo* receptor occupancy using radiolabeled technique. Lu AE60157 binds preferentially to rat brain regions with expected high 5-HT6 receptor density and the displacement of tracer upon pre-treatment is widely employed in the calculation of receptor occupancy for test compounds. In the present investigation, receptor occupancies of selective 5-HT6 antagonists were tested using liquid scintillation counter and mass spectrometry based techniques employing [3H] Lu AE60157 and Lu AE60157 as tracers, respectively. For comparison of receptor occupancy, treatment time for 5-HT6 antagonists was kept identical in both techniques and dose response curves were generated upon pre-treatment. Non- radiolabeled Lu AE60157 was used a tracer in the mass- spectrometry based experiments and was administered at a dose of 0.3 μg/kg, i.v. and 30 min as a sacrifice time. Tritiated Lu AE60157 was used as a tracer in radiolabeled

experiments and was dosed at 10 μCi/ rat, i.v. using 15 min as a sacrifice time. Receptor occupancy in the striatum as a 5-HT6 receptor rich region was determined against the cerebellum as a receptor null region using specific binding method. Dose dependent receptor occupancy of test compounds was obtained with non- labeled and [3H] Lu AE60157 tracer. The ED50 values for tested 5-HT6 antagonists were comparable across both mass spectrometry and radiolabeled techniques. Results from the present investigation justify the use of mass spectrometry based method for rapid screening of new chemical entities during early stages of drug discovery.

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Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.10/I11

Topic: C.19. Drug Discovery and Development

Support: USPHSG #NS084261

Title: A novel aryl 2-cylcopropylamine improves motor impairment in a rat model of Parkinson's disease

Authors: *B. D. BRADARIC[1,2], D. MCCAFFERTY[4], F. NWOGBO[4], K. ALSER[4], L. OLIVERE[4], N. STEDER, III[3], A. L. PERSONS[1,2], T. C. NAPIER[1,2];
[1]Pharmacol., [2]Ctr. for Compulsive Behavior & Addiction, [3]Med. Lab. Sci., Rush Univ. Med. Ctr., Chicago, IL; [4]Chem., Duke Univ., Durham, NC

Abstract: Parkinson's disease (PD) is a common neurodegenerative disease. Clinical symptoms of PD include resting tremor, rigidity, slowness of voluntary movement, and postural instability, which are associated with loss of dopamine in the nigrostriatal pathway. A treatment for PD is dopamine replacement with L-3,4-dihydroxyphenylalanine (L-DOPA); however, chronic treatment can result in abnormal involuntary movements (AIMs) called dyskinesias. Thus, the development of novel compounds that exhibit motor efficacy without the unwanted side effects will be useful for the treatment of PD. Toward that end, the McCafferty laboratory synthesized and screened over 15 novel aryl 2-cylcopropylamine compounds in a dopamine transporter knockout/dopamine depleted mouse model of PD for improved locomotor activity. Two of the most promising novel compounds were then tested in the Napier laboratory for postural instability and dyskinesias in the unilateral 6-hydroxydopamine rat model of PD. Following

administration of the aryl 2-cyclopropylamine compounds (3-12 mg/kg, ip) or L-DOPA (3 mg/kg, ip; the positive control), lesioned rats were tested for postural instability using the forelimb adjusted step task and evaluated for dyskinesias using locomotor, axial dystonia, and limb dyskinesia parameters. The safety profile for the aryl 2-cyclopropylamine compounds was also determined. Lesions were confirmed by tyrosine hydroxylase immunoreactivity. Lesioned-induced stepping deficits improved following acute administration of the aryl 2-cyclopropylamine compounds. However, higher doses (9-12 mg/kg) resulted in adverse events, including tremors, catalepsy and in some rats, seizures. To determine if motor efficacy could be retained with diminished toxicity, a subthreshold dose (3-0.5mg/kg, ip) of the most promising aryl 2-cyclopropylamine compound was co-administered with a subthreshold dose of L-DOPA (3 mg/kg). Combination treatment showed stepping improvement compared to supra-threshold L-DOPA treatment; however, the combination also resulted in AIMs. Taken together, these data indicate therapeutic potential for modified aryl 2-cylcopropylamine small molecules in PD, but suggest that additional derivatives to the ones tested here will need to be developed for use as adjunct treatment with L-DOPA.

Disclosures: **B.D. Bradaric:** None. **D. McCafferty:** None. **F. Nwogbo:** None. **K. Alser:** None. **L. Olivere:** None. **N. Steder:** None. **A.L. Persons:** None. **T.C. Napier:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.11/I12

Topic: C.19. Drug Discovery and Development

Support: AHA 12SDG8170005

Title: Induction of phase II detoxification enzymes through Nrf2 pathway provides protective effects in cerebral ischemic stroke

Authors: ***P.-C. KUO**[1], B. A. SCOFIELD[1], D. A. BROWN[2], J.-H. YEN[1];
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Abstract: Stroke is a leading cause of death in the world and cerebral ischemic stroke is composed of over 80% of all stroke cases. During cerebral ischemia reactive oxygen species (ROS) is produced in brain tissue, which can then initiate numerous signaling pathways and cause oxidative stress and inflammatory response. Studies suggest that both oxidative stress and

inflammatory damage are essential pathological mechanisms in cerebral ischemia. To date, recombinant tissue plasminogen activator (tPA) is the only available therapy for the treatment of ischemic stroke; however, the treatment does not prevent ischemia-induced oxidative stress and reperfusion-mediated inflammation in ischemic brains. Therefore, the development of new anti-oxidant and anti-inflammatory therapies for the treatment of ischemic stroke is necessary and urgent. Nuclear factor erythroid 2-related factor 2 (Nrf2), a major regulator of oxidative responses, is a key transcription factor which induces a wide set of anti-oxidant enzymes and phase II detoxification enzymes, including heme oxygenase 1 (HO-1) and NAD(P)H dehydrogenase, quinone 1 (NQO1). In addition, Nrf2 exerts an anti-inflammatory effect through downregulating NF-κB expression that would subsequently suppress inflammatory mediators in ischemic brains. Our lab has identified a small molecule (BY-23) with a property of inducing Nrf2 activation, and we evaluated its therapeutic potential for the treatment of ischemic stroke in the animal model of transient middle cerebral artery occlusion/reperfusion (MCAO/R). Our results show BY-23 treatment not only ameliorated neurological deficits in ischemic stroke mice but dramatically reduced infract size in ischemic brains. We also observed significant reduction of CNS infiltrating cells including neutrophils and monocytes in the ischemic brains of BY-23-treated MCAO mice when compared to those of vehicle-treated MCAO mice. At the molecular level, BY-23 treatment enhanced the expression of anti-oxidant enzymes HO-1 and NQO1 in the ischemic brains of MCAO mice. The effect of BY-23 on the induction of anti-oxidant associated genes was confirmed in primary microglia. In the presence of BY-23, the expression of Nrf2, HO-1, NQO1, and glutamate-cysteine ligase, catalytic subunit (GCLC) was significantly upregulated in LPS-activated microglia. Moreover, the expression of inflammatory cytokine IL-1β, IL-23, and IL-12 in activated microglia was inhibited by BY-23. Altogether, our results suggest that BY-23 confers a protective effect against ischemic stroke through its anti-oxidant and anti-inflammatory properties.

Disclosures: **P. Kuo:** None. **B.A. Scofield:** None. **D.A. Brown:** None. **J. Yen:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.12/I13

Topic: C.19. Drug Discovery and Development

Support: CAPES

FAPESP

Title: Glutamate transporter activator Parawixin-10 present neuroprotective activity in rats submitted to endothelin-1 experimental model of Stroke

Authors: *J. L. LIBERATO[1,2], J. MARIN-PRIDA[3], T. BRONHARA[4], M. V. B. CELANI[5], L. GOBBO, Neto[7], N. P. LOPES[7], W. F. SANTOS[6];
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Abstract: Excitotoxicity in Stroke is mainly triggered by exacerbated glutamate (Glu) release, which is worsened when it is followed by reperfusion. Excessive extracellular Glu leads to necrotic and apoptotic signaling pathways onset. Therefore, compounds that enhance Glu uptake represent promising tools for providing an efficient protection against stroke injury through increase of Glu clearance. Parawixin-10 (Pwx10), presents a potent activity in enhancing glutamate uptake in rat cerebrocortical synaptosomes. Besides that, it presents anticonvulsant effects on acute models of chemically induced seizures. Therefore, the present study aim to evaluate the neuroprotective effects of Pwx-10 on motor cortex and striatum, as well as evaluating neurological deficits after treatment in experimental model of Stroke. Wistar rats were stereotaxically implanted with a steel guide-cannula into the piriform cortex, to access Middle Cerebral Artery (MCA) and in right ventricule to drug delivery. Occlusion of the right MCA was induced in awake rats by endothelin-1 (ET-1; 400 ρmol in 4 μL) injection. SHAM animals were injected with saline (in 4 μL). Neurologic deficit (inclusion parameters) were evaluated 10 minutes after ischemia by Open field test (OFT) (counterclockwise circling, clenching, or failure to extend the contralateral forelimb) and Tail Suspension Test (TST). Treatment was conduct 30 min from behavioral analyzes, with Pwx10 (2 μg/μL; 1μL) or Saline 0.9% (1 μL) injections. Neurologic deficit were assessed by OFT, TS additionally with Adhesive Test (AT), after 3 days post stroke. Shortly afterwards, rats were decapitated or transcardially perfused. Neuronal death in cortical and striatal infarcted areas was evaluated by quantification of Fluoro-Jade C (FJC) positive cells and triphenyltetrazolium chloride (TTC) method, respectively. Treatment with Pwx10 improved neurological function in and in OFT ($n=6$, $p<0.0001$), TST tests ($n=6$, $p<0.001$) and AT test ($n=6$, $p<0.001$). Noteworthy, Pwx10 was efficient in reducing infarct volume area ($n=$, $p<0.0025$) in TTC method and decreasing FJC-positive cells in cortex ($n=7$, $p<0.0001$) and striatum ($n=7$, $p<0.0001$). Considering that Pwx10 is one of very few compounds that stimulate promptly the activity of glutamate clearance, our findings suggest that this neuroprotective potential might represent a promissory drug in stroke therapeutic

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Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

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Program#/Poster#: 55.13/I14

Topic: C.19. Drug Discovery and Development

Support: TEVA Grant

Title: Action of copaxone at the mitochondrial level in EAE

Authors: *V. K. NIMMAGADDA[1,2], P. GUDA[1], C. BEVER[1,2], T. MAKAR[1,2];
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Abstract: Multiple sclerosis (MS) is characterized by various pathophysiological molecular alterations, such as ionic homeostasis, overproduction of reactive oxygen and nitrogen species. Oxidative and nitrosative stress leads to the induction of apoptosis. All these events comprise several fundamental neuronal functions. These molecular changes seem to have as a common feature the malfunctioning of mitochondria which has led to the “mitochondrial hypothesis” of axonal degeneration in MS. Glatiramer acetate (Copaxone) is one of the drug used for MS. The therapeutic effects of this drug in the treatment of MS are thought to be via immunomodulation and neuroprotection. However, its role in mitochondrial protection and regulating energy metabolism is not well studied. In this study, we explored the effects of Copaxone on mitochondrial protection and balancing energy metabolism in EAE. Treatment with Copaxone (20 mg/kg, ip, daily for 30days) in EAE mice showed an inhibition of clinical severity and inflammation compared to EAE mice. Quantitative Western blot analysis and immunohistochemical staining revealed an increase in PGC-1α, an upstream regulator of transcription factors involved in mitobiogenesis, and several of its downstream targets (NRF2, TFAM) in Copaxone- treated EAE animals compared to EAE mice. This correlated with an increase in protein expression of citrate synthase, a mitochondrial marker and increase in protein reflecting mitochondrial fusion (as determined by detecting Mfn2 and OPa-1). We also found inhibition of mitochondrial fission (as determined by detecting Drp-1, Fis-1) in Copaxone treated EAE animals compare to EAE animals. SIRT1 and SIRT3 also significantly elevated after Copaxone treatment in EAE mice. These enzymes play a key role in mitochondrial protection. Collectively, these results revealed an increase in mitochondrial function, integrity and

biogenesis after Copaxone treatment in EAE mice. Furthermore, our findings suggest that Copaxone exerts a regulatory effect on mitochondria through SIRT1 and SIRT3 mechanisms involving mitochondria and cytosolic proteins.

Disclosures: V.K. Nimmagadda: None. **P. Guda:** None. **C. Bever:** None. **T. Makar:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.14/I15

Topic: C.19. Drug Discovery and Development

Support: Study supported by Project LS 10-32, NFB Austria

Title: Lack of additive effect of D-cycloserine and cerebrolysin to inhibit formation of kynurenic acid in rat liver homogenate

Authors: *H. BARAN, B. KEPPLINGER;
Karl Landsteiner Res. Inst. Mauer, Mauer-Amstetten, Austria

Abstract: Background and aim of study: Effect of Cerebrolysin (CER) and D-cycloserine (D-CS) in the treatment of dementia and brain injury has been reported. Kynurenic acid (KYNA), a metabolite of tryptophan, is an antagonist of the glutamate ionotropic EAA and of the 7α-nicotine-cholinergic receptors and its involvement in the impairment of memory and cognition have been suggested. Recently, we demonstrated the ability of CER and D-CS to lower the activities of kynurenine aminotransferase I, II, III (KAT I, II, III), enzymes synthesising KYNA (Baran H and Kepplinger B, Eur. Neuropsychopharmacol 2008, 2014). Furthermore, we have shown that lowering of KATs activities by D-CS is primary due to lowering of pyridoxal-5-phosphate levels (Baran H and Kepplinger B Soc. Neurosci. Abstr 2014). Since an increased kynurenine metabolism has been documented in several brain pathologies, we investigated if the effect of CER or D-CS to lower KYNA synthesis is additive. In this study we investigated if CER also affects pyridoxal-5-phosphate levels. Methods The activities of the KAT II in rat liver homogenate were analysed in the presence of CER or D-CS or mixture of CER and D-CS and in the absence, as control. KAT II activities were measured in the presence of 1 mM pyruvate and 100 µM L-kynurenine and synthesized KYNA as well pyridoxal-5-phosphate levels were determined by HPLC method. The statistical analysis was carried out by one-way-ANOVA and Student's-T-Test. The study was performed according to the ethical regulations of the government of Austria. Results CER and D-CS reduce KAT II activity significantly of rat liver

homogenate, by 80 % of CO and 30 % of CO, N=8; P<0.01, respectively, but the mixture of CER and D-CS did not additively affect the inhibition of KYNA formation (30 % of CO, N=8), at least *in vitro* conditions. CER does not affect pyridoxal-5-phosphate levels under present experimental conditions. Discussion The mechanism of CER and D-CS to lower KYNA levels in rat liver homogenate seems to be different. Furthermore, revealed data indicate that the inhibitory effect on KAT II activity of both agents was non additive in the incubation medium. We observed that the effect of a CER and D-CS mixture was similar to the effect of D-CS alone. CER does not affect the levels of pyridoxal-5-phosphate levels under experimental conditions using rat liver homogenate. Since both drugs are used for the treatment of dementia and cognition further work needs to be done to exclude interaction between both pharmacological agents.

Disclosures: H. Baran: None. **B. Kepplinger:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.15/I16

Topic: C.19. Drug Discovery and Development

Support: T32-NIH Training Grant in Sleep Medicine Neurobiology

Title: Identification of hypothalamus biomarkers in orexin knockout and ataxin mouse models of narcolepsy: a proteomic quantitative approach

Authors: *S. AZZAM[1], P. J. SHIROMANI[3], P. FENG[2], K. P. STROHL[4];
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Abstract: Human narcolepsy is a chronic brain disorder characterized by excessive daytime sleepiness with a dysregulation of the sleep-wake cycles. Narcolepsy is linked to the loss of orexin neurons producing orexin neuropeptides. Although the proximate cause of orexin neuron cell loss is unknown, a neurodegeneration process occurs. Two mouse models of narcolepsy have been used extensively to test drugs: orexin-Knockout (KO, orexin gene knockout), and orexin/ataxin-3 (Atx, genetic ablation of orexin neurons). The hypothesis was that models might have different functional consequences depending on how orexin loss is achieved, and to this end we employed label-free quantitative proteomics and pathway analysis. In this study, we

examined the hypothalamus proteome of KO, Atx and appropriate wild type (WT=C57BL/6J) mice. Each sample was subjected to in-solution digestion and analyzed by LC-MS/MS using an LTQ-Orbitrap Elites mass spectrometer. Differential quantification of peptides was performed using Rosetta Elucidator (FDR <2%). ANOVA was performed comparing peptide intensities across groups. Protein fold changes were determined by dividing the median protein intensity for each peptide in each study group. Differentially expressed proteins (p-value ≤ 0.05, fold-change ±1.5, and a minimum of 2 peptides per protein) were imported into Ingenuity Pathway Analysis (IPA). Analysis of 16 samples identified 2282 non-redundant proteins; only 39 proteins were statistically different across the groups. From these 39 proteins, 12 were specific to Atx/WT, 3 were specific to KO/WT, 10 were shared between KO/WT and Atx/WT, and 2 were shared between Atx/WT and Atx/KO. The majority of proteins, including MAO, DARPP-32, SIRT2, SNAP-25, COX5A, PKM, Hbb-b1, NFs, DPYSL3 and CKB, are associated with neurodegeneration, and mitochondrial respiratory and axonal dysfunction. IPA analysis revealed dysregulated pathways including glycolysis and mitochondrial dysfunction, as well as dopamine signaling, melatonin degradation, and glutamate degradation not expected by known orexin pathway action. Additionally, IPA analysis revealed a number of less specific but significant networks (p-value ≤0.05); including those “cellular assembly and organization”, “neurological disease”, and “behavior” networks. No protein differences were uniquely shared across all three groups. Taken together, peptide identification in this analysis found several dysregulated pathways, some like MAO which is targets for current narcolepsy therapy and other targets for circadian, excitatory circuit, and cell cycle deficits.

Disclosures: **S. Azzam:** None. **P.J. Shiromani:** None. **P. Feng:** None. **K.P. Strohl:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.16/I17

Topic: C.03. Parkinson’s Disease

Support: Swedish Research Council

Swedish Parkinson Foundation

Swedish Alzheimer foundation

AstraZeneca research grant

Swedish Brain Power

Title: Increased myeloperoxidase expression in brain areas affected by neurodegeneration in Alzheimer's and Parkinson's disease

Authors: S. GELLHAAR[1], D. SUNNEMARK[2], H. ERIKSSON[2], L. OLSON[1], *D. GALTER[1]; [1]Karolinska Institutet, Dept. of Neurosci., Stockholm, Sweden; [2]AstraZeneca R&D, Södertälje, Sweden

Abstract: Myeloperoxidase (MPO), a key enzyme in inflammatory processes, has been implicated in neurodegeneration by genetic and histological findings. We studied MPO expression in Parkinson's disease (PD) and Alzheimer's disease (AD) patients and neurologically unaffected control cases and in two rodent PD models, the 6-OHDA rat model and the MitoPark mouse. In human brain tissue MPO immunoreactivity (ir) was detected in monocytes in capillaries, perivascular macrophages and amoeboid microglia in the brain parenchyma, and no co-localization with GFAP ir was observed. In brain regions highly affected by PD such as midbrain, caudate and putamen we found a significant increase of MPO ir cells in parkinsonian brains as compared to control brains, whereas there was no such difference in cerebellum. MPO ir was not detected in neuronal cells or in occasional small beta-amyloid ir plaques in any region from PD or control cases. In frontal cortex of AD patients many MPO ir microglia / macrophages were detected together with plenty of MPO ir in extracellular plaques. In hippocampus of several AD cases MPO-like ir was observed in some pyramidal neurons, preferentially in the CA1 region, most likely unspecific binding to highly modified proteins. Neither the rapid dopamine (DA) depletion in the rat PD model, nor the slow degeneration of DA neurons in MitoPark mice induced expression of MPO in brain cells in any brain region. MPO mRNA was not detectable with radioactive in-situ hybridization in any human or rodent brain tissue and no enzymatic MPO activity was observed with antibodies recognizing hypochloric-acid modified proteins. Taken together we demonstrate a significant increase in numbers of MPO ir cells in brain regions affected by neurodegeneration in both PD and AD, indicating that higher MPO enzyme activity may be part of the pathological process.

Disclosures: S. Gellhaar: None. **D. Sunnemark:** A. Employment/Salary (full or part-time):; AstraZeneca R&D, Södertälje, Sweden. **H. Eriksson:** A. Employment/Salary (full or part-time):; AstraZeneca R&D, Södertälje, Sweden. **L. Olson:** None. **D. Galter:** 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.; AstraZeneca R&D, Södertälje, Sweden.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.17/I18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSF GRFP

Kavli Institute for Neuroscience

NIH

Falk Medical Research Trust

Title: Deletion of Nogo Receptor 1 enhances fear extinction in adulthood

Authors: *S. M. BHAGAT[1], B. S. MCEWEN[3], J. R. TAYLOR[2], S. M. STRITTMATTER[2]; [1]Cell. Neuroscience, Neurodegeneration and Repair, [2]Yale Univ., New Haven, CT; [3]Rockefeller Univ., New York, NY

Abstract: In adult rodents, fear conditioning produces a lifelong fear memory that cannot be permanently repressed or erased, even following extinction training. This contrasts with what has been reported in juvenile rodents, which exhibit fear erasure following extinction training. The formation of myelin and perineuronal nets, which are extracellular matrices rich of chondroitin sulfate proteoglycans, coincide with the critical period for fear erasure. We have found that Nogo Receptor 1 (NgR1), a neuronal receptor for myelin-associated inhibitors that block neuronal sprouting and cortical plasticity in adulthood, significantly inhibits plasticity during fear and extinction learning. We have found that adult male NgR1 knockout (KO) mice, in contrast to wild-type (WT) controls, show a significant enhancement in fear extinction recall and no spontaneous recovery or fear renewal, which are behavioral assays to measure recall of the original fear memory. We have used genetic and viral-manipulations to explore the temporal, regional, and cell-specific role of NgR1. In conclusion, NgR1 plays a pivotal role in limiting extinction learning in adulthood, which could help enhance our current insight into neuropsychiatric diseases, such as anxiety disorders and post-traumatic stress disorder (PTSD).

Disclosures: S.M. Bhagat: None. **B.S. McEwen:** None. **J.R. Taylor:** None. **S.M. Strittmatter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cofounder of Axerion Therapeutics, seeking to develop PrP- and NgR-based therapeutics.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.18/I19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NFSC 31100919

NFSC 81371469

NFSZJ LY15090010

Title: Elevation of peripheral BDNF promoter methylation predicts the risk of Alzheimer's disease

Authors: *L. CHANG[1], H. JI[2], G. LIU[2], S. DUAN[2], Q. WANG[2];
[1]Zhejiang Provincial Key Lab. of Pathophysiol, Zhejiang, China; [2]Ningbo Key Lab. of Behavioral Neuroscience, Sch. of Medicine, Ningbo Univ., Ningbo, China

Abstract: Brain derived neurotrophic factor (BDNF) can protect neurons from various attacks and thus play an important role in various mental diseases such as Alzheimer's disease (AD). Previous study has shown a significantly higher BDNF methylation in AD brain tissues. The aim of our study was to assess whether BDNF promoter methylation in peripheral blood was able to predict the risk of AD. DNA was extracted from the blood samples of 44 AD patients and 62 age- and gender-matched controls. Using the bisulphite pyrosequencing technology, we evaluated 4 CpG sites in BDNF promoter. Our results showed that BDNF methylation in the blood was significantly higher in AD cases than controls (CpG1: $p = 0.021$; CpG2: $p = 0.002$; CpG3: $p = 0.007$; CpG4: $p = 0.005$; average methylation: $p = 0.004$). BDNF methylation in the blood was shown as a good predictor of AD risk (CpG1: area under curve (AUC) = 0.617, $p = 0.041$; CpG2: AUC = 0.692, $p = 0.001$; CpG3: AUC = 0.663, $p = 0.004$; CpG4: AUC = 0.649, $p = 0.009$; average CpG: AUC = 0.657, $p = 0.006$). In addition, BDNF promoter methylation was shown to be significantly correlated with the levels of alkaline phosphatase (ALP), glucose, Lp(a), ApoE and ApoA in males (ALP: $r = -0.308$, $p = 0.042$; glucose: $r = -0.383$, $p = 0.010$; Lp(a): $r = 0.333$, $p = 0.027$; ApoE: $r = -0.345$, $p = 0.032$;), ApoA levels in females ($r = 0.362$, $p = 0.033$) and C Reactive Protein (CRP) levels in both genders (males: $r = -0.373$, $p = 0.016$; females: $r = -0.399$, $p = 0.021$). Our work was the first one to show that elevation of peripheral BDNF methylation was able to predict the risk of AD.

Disclosures: **L. Chang:** None. **H. Ji:** None. **G. Liu:** None. **S. Duan:** None. **Q. Wang:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.19/I20

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIEHS R01ES020715

BrightFocus A2011084

GHR Foundation

NCATS UL1 TR000135

Title: Impact of environmental toxicants on mitochondrial dynamics and function in neurons

Authors: L. ZHANG, B. GATENO, *E. TRUSHINA;
Dept. of Neurol., Mayo Clin., Rochester, MN

Abstract: Mitochondrial dysfunction is implicated in the etiology of multiple neurodegenerative diseases including Alzheimer's Disease (AD). Since most of neurodegenerative disorders are sporadic, environmental exposure may play a major role in initiating the disease via affecting mitochondrial function. Indeed, many compounds commonly used in agriculture selectively target individual components of mitochondrial electron transport chain (ETC). We tested whether the exposure to multiple toxicants that target different ETC complexes leads to common downstream effects on cellular energetics, mitochondrial dynamics and function in primary cortical neurons from wild type mice. We examined the exposure to rotenone (ROT, pesticide), paraquat (PQ, herbicide), maneb (MB, fungicide), atpenin (A5, fungicide) and 3-nitropropionic acid (3NA) using two experimental paradigms, an acute (2 h) and prolonged (72 h) exposure at sub-lethal levels, which mimics occupational hazard and daily life exposure. We found that acute exposure to all toxicants rapidly depleted cellular ATP levels. These changes were especially significant when neurons were exposed to rotenone, a specific inhibitor of mitochondrial complex I. However, an acute exposure had little impact on mitochondrial respiration or function assessed in intact neurons using a Seahorse Extracellular Flux Analyzer. In contrast, prolonged exposure to toxicants along with a significant reduction in cellular ATP levels induced substantial energetic crisis. Specifically, toxicant-treated neurons displayed significant loss (>50%) of spare respiratory capacity, a measure of mitochondrial ability to produce energy under stress conditions. Both acute and prolonged exposure inhibited mitochondrial axonal trafficking and significantly increased the number of stationary mitochondria measured using time-lapse imaging in live neurons. Unexpectedly, increased production of reactive oxygen species was observed under both conditions only in neurons treated with ROT, NB and A5 but not in cells

exposed to PQ or 3NA. Our findings suggest that similar to what we observed in neurons from multiple animal models of AD, exposure to environmental toxicants, regardless of their origin, in the first place depletes cellular energy reserve and inhibits axonal trafficking where loss of bioenergetics occurs only after a prolonged exposure.

Disclosures: **L. Zhang:** None. **B. Gateno:** None. **E. Trushina:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.20/I21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NFSC 31100919

NFSC 81471398

NFSZJ LY15090010

Title: Elevated OPRD1 promoter methylation in Alzheimer’s disease patients

Authors: ***Q. WANG**[1], H. JI[2], L. CHANG[2], G. LIU[2], S. DUAN[2];
[2]Ningbo Key Lab. of Behavioral Neuroscience, Zhejiang Provincial Key Lab. of Pathophysiol, [1]Ningbo University, Med. Sch., Zhejiang, China

Abstract: DNA methylation was reported to be involved in Alzheimer’s disease (AD), a most common neurodegenerative disorder in the elderly. In the current study, we tested the levels of OPRD1 promoter CpG sites (CpG1, CpG2, and CpG3) among 51 AD cases and 63 controls by bisulphite pyrosequencing technology. Our results showed that significantly higher CpG3 methylation was found in AD cases than controls (CpG3: p = 1.68E-7, adjusted p = 0.001). Significant associations were found between the CpG3 methylation and a series of phenotypes. Subsequent luciferase assay showed that DNA fragments of OPRD1 promoter tended to have higher expression of the reporter gene (CpG1-3 containing DNA fragment: fold = 1.923, p = 0.0883; CpG1-3 nearby DNA fragment: fold = 2.221, p = 0.0114). In summary, our results suggested that OPRD1 promoter hypermethylation increased the risk of AD through the regulation of gene expression.

Disclosures: **Q. Wang:** None. **H. Ji:** None. **L. Chang:** None. **G. Liu:** None. **S. Duan:** None.

Poster

055. Neurodegeneration Drug Discovery: AD, PD, and Gene Therapy

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 55.21/I22

Topic: C.19. Drug Discovery and Development

Title: Modeling neurological disorders using human induced Pluripotent Stem cells-derived neurons

Authors: *B. B. DOROTHEE[1], E. GRAS LAVIGNE[1], L. THON[1], C. BADJA[2], M. OUAMER[1], R. STEINSCHNEIDER[1], F. MAGDINIER[2];
[1]Neuron Experts, Marseille, France; [2]INSERM UMR_U910 GMGF, Marseille, France

Abstract: Neurodegenerative diseases are a major health problem. About 20-30 million people worldwide suffer from Alzheimer disease and this number is predicted to quadruple by 2050. Unfortunately, very few treatments are available. New therapeutic strategies allowing to diminish symptoms but also to cure are needed. Tt is now essential to develop appropriate tools to better understand the underlying molecular mechanisms of these diseases and to create relevant models of these pathologies. Animal models and corresponding cellular models have given important insight in the understanding of disease mechanisms and have greatly facilitated the assessment of new therapeutic strategies. However, these models also display major limitations such as the inability to reproducibly mimic human pathology and the often unpredictable effect of test compounds on humans. The use of human induced Pluripotent Stem cells (iPS) derived neurons is an attractive approach to get closer to human physiological conditions and to propose relevant *in vitro* cellular models to be used for drug discovery through *in vitro* high content screening. For these reasons, we attempt to produce two kinds of neurons for two major neurodegenerative diseases. Firstly, we differentiated human iPS cells into cortical neurons in order to develop Alzheimer's disease cellular models. By employing phenotypic and molecular markers, we characterize the iPS-derived neurons before testing them in widely used cell models of exitotoxic stress and β-amyloid insult. Secondly, we produced dopaminergic neurons in order to develop Parkinson's disease cellular models. We confirmed their molecular identity by immunolabeling and submitted them to mimicking models of Parkinson's disease such as: impairment of mitochondria by MPP+ intoxication, oxidative stress (6-OHDA injury) or α-synuclein intoxication. Finally, we quantified the responsiveness of the generated human dopaminergic neurons to these toxins and compared them to our rodent cell models. These new *in vitro* cellular models, based on human neurons appeared to be more predictive and relevant models than rodent ones and could be used for the evaluation of therapeutic compounds.

Disclosures: **B.B. Dorothee:** None. **E. Gras Lavigne:** None. **L. Thon:** None. **C. Badja:** None. **M. Ouamer:** None. **R. Steinschneider:** None. **F. Magdinier:** None.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.01/I23

Topic: C.19. Drug Discovery and Development

Title: *In vivo* preclinical efficacy of Nav1.7 inhibitors in pain and olfactory models

Authors: *R. SANOJA, F. ZHAO, M. HOLAHAN, C. WINKELMANN, C. BURGEY, A. HOUGHTON;
Merck and Co., Inc., West Point, PA

Abstract: Pain is a frequent debilitating feature reported in peripheral neuropathies with involvement of small nerve (Aδ and C) fibers (Faber et.al., J. Peripher. Nerv. Syst.; 2014). Voltage-gated sodium channels are responsible for the generation and conduction of action potentials in the peripheral nociceptive neuronal pathway where voltage-gated sodium channel 1.7 ($Na_V1.7$) has a critical role. Human loss-of-function (LOF) mutations in the SCN9A gene, which encodes $Na_V1.7$, result in congenital indifference to pain and anosmia (Ruitenberg et. al., Channels; 2012). To test the ability of small molecule inhibitors of $Na_V1.7$ to recapitulate the human phenotype, we examined the efficacy of two Merck $Na_V1.7$ inhibitors (Compound A and B) *in vivo* assays that are preclinical surrogates for either neuropathic pain (ectopic firing in rats) or olfaction (functional MRI olfactory assay in non-human primates). *Sprague Dawley Rats' electrophysiology protocol:* A lumbar laminectomy 3-7 days after CCI (chronic constriction injury model) induction was performed to expose the left L5 dorsal root, cut at the spinal cord level, teased and immersed in a mineral oil pool. The ipsilateral sciatic nerve was exposed and freed from adherences to allow electrical stimulation and fiber characterization. Only single teased Aδ fibers with ongoing irregular spontaneous activity were chosen to study further. *NHP functional olfactory magnetic resonance imaging protocol:* Rhesus monkeys were exposed cyclically to an odor (Isoamyl acetate ~ 2800 ppm), for one minute followed by three minute recovery, for an hour while continuous fMRI images were taken. For both protocols, long baselines -no stimulation- were always done followed by iv compounds infusion (10 min for rats, 60 min for NHP) and vehicle was 30% captisol in all cases. Both protocols were also done under isoflurane anesthesia. *Results:* Compound A regularized and decreased Aδ ectopic firing activity in CCI induced animals and Compound B significantly impaired the NHP fMRI signal, both in a

dose dependent manner. These results demonstrate that small molecule inhibitors of $Na_V1.7$ can replicate the clinical phenotype of human LOF patients. Our findings corroborate the critical role of $Na_V1.7$ channels in neuropathic spontaneous pain and anosmia, and provide pre-clinical efficacy for our $Na_V1.7$ inhibitors.

Disclosures: **R. Sanoja:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **F. Zhao:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **M. Holahan:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **C. Winkelmann:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **C. Burgey:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **A. Houghton:** A. Employment/Salary (full or part-time):; Merck & Co., Inc..

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.02/I24

Topic: C.19. Drug Discovery and Development

Title: Effect of tumor necrosis factor inhibition on central nervous system structure and function in rheumatoid arthritis patients

Authors: ***M. BJORNSDOTTER**[1], S. TÖYRÄ SILVERSWÄRD[2], L. LEIFSDOTTIR[2], H. BACKLUND-WASLING[1], H. OLAUSSON[3], M. BOKAREWA[2];
[1]Clin. Neurophysiol., Univ. of Gothenburg, Goteborg, Sweden; [2]Dept. of Rheumatology and Inflammation Research, Sahlgrenska Univ. Hospital, Univ. of Göteborg, Göteborg, Sweden; [3]IKE, Linkoping Univ., Linkoping, Sweden

Abstract: Rheumatoid arthritis (RA) is an autoimmune disorder characterized by chronic joint inflammation. Inhibition of tumor necrosis factor (TNFi) has emerged as an effective treatment reducing systemic inflammation. In the short-term, TNFi reverses hyperactivity in pain circuits (Hess et al. PNAS, 2011:108; Rech et al. Arthritis Rheum, 2013:65). Any long-term effects of TNFi on the central nervous system are unclear, however. We therefore studied the effect of TNFi on brain structure and function by examining i) group differences between TNFi treated and untreated RA patients, and, ii) effects of duration in TNFi patients. We collected anatomical and functional MRI scans in 15 female patients (mean age 55 years, std 8.6 years) with established RA (mean duration 11.4 years, std 7.7 years). Eight patients had long-term TNFi medication (mean duration 56, range 11-100 months). The fMRI paradigm involved alternating blocks of painful compression of the left metacarpophalangeal II joint and rest. Data processing

was performed SPM8 and VBM8. We conducted general linear modeling analyses, including age and duration as covariates, within an anatomically defined mask comprising structures associated with pain and sensorimotor processing (Hess et al. PNAS, 2011:108; Smallwood et al. J Pain, 2013:14). Results were assessed at uncorrected $p < 0.01$, corrected for multiple comparisons using AlphSim with 1,000 iterations. Patients treated with TNFi had larger gray matter volume and higher pain responses in sensorimotor regions (post/pre-central gyrus) and the anterior cingulate cortex ($p < 0.01$, $\alpha < 0.05$). Duration of TNFi treatment correlated positively with gray matter volume of the postcentral gyrus, and negatively with gray matter volume in a range of areas including the anterior cingulate cortex ($p < 0.01$, $\alpha < 0.05$). Duration of TNFi treatment correlated negatively with the induced pain responses of the anterior cingulate cortex ($p < 0.01$, $\alpha < 0.05$). Our results suggest that TNFi treatment induces structural and functional central nervous system alterations also in the long term, corroborating previous findings from studies of short-term effects of medication (Hess et al. PNAS, 2011:108). Moreover, our findings support the notion that patients who are hyperresponsive to painful stimuli benefit from treatment with TNFi (Rech et al. Arthritis Rheum, 2013:65).

Disclosures: **M. Bjornsdotter:** None. **S. Töyrä Silverswärd:** None. **L. Leifsdottir:** None. **H. Backlund-Wasling:** None. **H. Olausson:** None. **M. Bokarewa:** None.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.03/I25

Topic: C.19. Drug Discovery and Development

Title: Analgesic and anti-inflammatory activities of water extract of Euphorbia thymifolia (Euphorbiaceae)

Authors: *E. NGO BUM, K. NGO NGIMOUT, J. K. S. NJAPDOUNKE, G. N. C. NKANTCHOUA, G. N. C. NKANTCHOUA;
Univ. Ngaoundere, Cameroon, Ngaoundere, Cameroon

Abstract: Euphorbia thymifolia (Euphorbiaceae) is a plant used in traditional medicine in the Littoral Region in Cameroon to treat pain and inflammation. This study evaluated the analgesic and anti-inflammatory effects of the whole plant of E. thymifolia. Mice or rats were divided into 5 groups of 5 animals. One negative control group, one positive control group and three groups treated with the macerate of the plant. Intraperitoneal administration of acetic acid 1% was used to induce abdominal contractions in mice. Tail immersion in the water heated to 55 ± 0.5°C was

used to induce pain in mice. Inflammation was induced by injecting 0.1 ml of carrageenan 1% in the footpad of the right posterior leg of the rats. Chronic inflammation was induced by the implantation of cotton pellets (sterilized at 69°C) in the left axilla of rats. The macerate was administered orally. The analysis of variance (ANOVA) two-way, followed by Turkey as post hoc test was used for statistical analysis using XLSTAT 2007. E. thymifolia at the dose of 75 mg/kg and morphine at the dose of 10 mg/kg inhibited 65.7 and 88.3% of abdominal contractions induced by acetic acid, respectively. When pain was induced by the immersion of the rat tail in heated water, the administration of E. thymifolia (75 mg/kg) and morphine (10 mg/kg) resulted to a maximum inhibition of 82.6 and 98.66% respectively, from the first 30 min and for 3 hours. In the third experiment, the edema of the footpad induced by carrageenan was inhibited by 63.63 and 90.9% by E. thymifolia (75 mg/kg) and dexamethasone (5 mg/kg), respectively. While the inhibition induced by the positive control dexamethasone was decreasing with time (54.55% 6h later), the inhibition induced by E. thymifolia was increasing (77% 6h later). The granuloma induction by implantation of cotton pellets in the axilla was inhibited by 74.3 and 73.4% by E. thymifolia (75 mg/kg), and by dexamethasone (5 mg/kg), respectively. These results show that water extract of E. thymifolia administered orally has analgesic and anti-inflammatory activities. The effectiveness of E. thymifolia could be due to alkaloids, flavonoids, tannins or saponins contained in the plant. These results justify the use of E. thymifolia in traditional medicine in Cameroon.

Disclosures: **E. Ngo Bum:** None. **K. Ngo Ngimout:** None. **J.K.S. Njapdounke:** None. **G.N.C. Nkantchoua:** None. **G.N.C. Nkantchoua:** None.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Training Grant GM008306

Mayday fund

Title: Oxaliplatin-induced neuropathic pain is prevented by A3 adenosine receptor agonists through spinally mediated mechanisms of action that engage IL-10 signaling

Authors: *C. WAHLMAN[1], K. JANES[1], K. JACOBSON[2], D. SALVEMINI[1];
[1]St. Louis Univ., Saint Louis, MO; [2]Natl. Inst. of Diabetes and Digestive and Kidney Dis., Bethesda, MD

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN), accompanied by chronic neuropathic pain, is a major dose-limiting side effect and cause of dose reduction for many anti-cancer agents. Oxaliplatin is one such chemotherapeutic in which approximately 35% of patients suffer from enduring neuropathic pain that lasts for months to years following treatment cessation severely limiting their quality of life. Currently, there are no effective treatments. Adenosine, an endogenous nucleoside, acts on four different G-protein coupled receptors: A1AR, A2AAR, A2BAR, and A3AR, of which A1AR and A2AAR have been investigated for their beneficial activities in models of neuropathic pain, but negative side effects limit their therapeutic potential. However, A3AR agonists are in phase II/III clinical trials for inflammatory disorders and as novel anti-cancer agents with good safety profiles. Our lab has shown that systemic administration of A3AR agonists prevents the development of oxaliplatin-induced neuropathic pain by attenuating spinal neuroinflammatory processes; A3AR agonists do not interfere with oxaliplatin's anti-cancer effects *in vitro*. Here we demonstrate that the spinal cord is an important site of action for the beneficial effects of systemic MRS5698, since spinal injection of an A3AR antagonist blocked the effects of systemic MRS5698. Spinal injection of MRS5698 given during oxaliplatin treatment abrogated the development of neuropathic pain as well. Noteworthy, the prevention of neuropathic pain by spinal MRS5698 was blocked by an intrathecal injection of an anti-IL-10 antibody but not its IgG control given just prior to MRS5698. These results suggest that MRS5698's preventative actions are dependent upon continuous IL-10 signaling within the spinal cord. IL-10 can be regulated in numerous ways, one of which is through miRNAs; this is under investigation. These results support A3AR agonists as a novel class of non-narcotic analgesics that are urgently needed for individuals currently suffering from CIPN.

Disclosures: C. Wahlman: None. **K. Janes:** None. **K. Jacobson:** None. **D. Salvemini:** None.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.05/I27

Topic: C.19. Drug Discovery and Development

Title: Effects of palmitoylethanolamide upon cyclooxygenase enzyme activity

Authors: *L. E. GABRIELSSON, C. FOWLER;
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Abstract: Department of Pharmacology and Clinical Neuroscience, Umeå University, SE-901 87 Umeå *N*-palmitoylethanolamide (PEA), belonging to the class of *N*-acetylethanolamines (NEAs), has been shown to have anti-inflammatory and analgesic effects. The mechanism behind these effects is not yet fully understood although it seems like the most prominent explanation is trough peroxisome proliferator-activated receptor alpha (PPAR-α). It has been reported that treatment of rats with PEA reduces the observed activity of cyclooxygenase (COX)[1], but it is not known whether this is a direct or indirect effect of the compounds. Numerous fatty acids have been shown to bind allosterically to COX-1 and -2 and alter their activity[2,3], raising the possibility that this also occurs with PEA. With an Oxygraph System we investigated the effects of PEA upon the COX-catalysed oxygenation of arachidonic acid (AA, COX-1 and -2) and 2-arachidonoylglycerol (2-AG, COX-2). PEA was used in concentrations of 1, 3, 10μM and COX substrates were used in concentrations of 2 and 10μM. We found that PEA has no direct effect upon COX-2 enzyme activity. However, an interaction was observed between PEA and COX-1 at 2 μM AA (but not 10 μM AA) where the initial velocity of the enzyme activity was increased ($P<0.05$). It is concluded that PEA has modest direct effects upon the catalytic activity of COX isoforms *in vitro*. References [1]Costa *et al.*, (2002) Br J Pharmacol [2]Yuan *et al*. (2009) J Biol Chem [3]Zou *et al.* (2012) J Lipid Res

Disclosures: L.E. Gabrielsson: None. **C. Fowler:** None.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.06/I28

Topic: C.19. Drug Discovery and Development

Title: AN363, a novel GABAA α2/3/5 receptor subtype selective positive allosteric modulator for treatment of chronic pain

Authors: D. AMRUTKAR, P. AHRING, T. DYHRING, *K. S. NIELSEN, T. JACOBSEN, J. LARSEN;
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Abstract: Chronic pain is still a largely unresolved medical condition. Effective pharmacological approaches need to target alterations in the nociceptive system that are

responsible for mediating the pain sensation and resulting disability. Reduction in inhibitory GABAergic control in dorsal horn neurons is believed to be a relevant aspect of the neuroplastic changes observed in inflammatory and neuropathic conditions. Based on this hypothesis, we here report on analgesic activity of a novel receptor subtype selective GABAA α2/3/5 positive allosteric modulator in preclinical models of pain. We have tested AN363 at doses of 1,3,10 and 30 mg/kg p.o., in Complete Freund's adjuvant (CFA,100 µg), formalin (5%) and capsaicin (10 µg) induced acute inflammatory pain models as well as chronic constriction injury (CCI) induced neuropathic pain model in male Sprague-Dawley rats. The minimum effective dose (MED) varied between 3 to 10 mg/kg across the models. In accordance with lack of activity at the GABAA α1 subtype, AN363 did not cause ataxia at the therapeutic doses, as measured in the rat rotarod test. By the use of an *in vivo* 3H-L-655,708 binding assay it was demonstrated that AN363 readily penetrates the brain in rats and occupies the benzodiazepine site with an ED50 of 4.5 mg/kg, p.o. in Sprague-Dawley rats, which is in good correspondence with the MED in pain models. AN363 has a half-life of more than 2h in rats and is highly stable in human hepatocytes. The robust therapeutic effect seen with AN363 in animal models of pain, together with its selective *in vitro* profile, suggest that it could have potential benefit in clinical treatment of chronic pain in humans with improved side effects profile compared to existing treatment. These findings indicate more selective targeting of pain pathways with subtype selective GABAergic drugs. The lead candidate, AN363, constitute a new and promising class of analgesics particularly suitable for treating chronic pain syndrome.

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Poster

056. Pain, Headache and Migraine

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Support: Leukemia and Lymphoma Society Translational Research Grant 6241-13

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Mayday Fund

Title: FTY720, a functional antagonist of sphingosine-1-phosphate receptor subtype 1, prevents bortezomib-induced neuropathic pain through modulation of spinal neuro-inflammation

Authors: *K. STOCKSTILL, K. JANES, D. SALVEMINI;
Pharmacol. and Physiological Sci., St. Louis Univ. Sch. of Med., Saint Louis, MO

Abstract: Bortezomib, a chemotherapeutic agent that is used to treat multiple myeloma and mantle cell lymphoma, can cause peripheral neuropathy in about 40% of patients. Bortezomib-induced peripheral neuropathy (BIPN) is often accompanied by chronic neuropathic pain and is one of the major reasons why patients either limit or discontinue their chemotherapy treatment. Currently, there are no effective therapies. Our lab has demonstrated that activation of the S1P-S1PR1 axis in the spinal cord (SC) is critical to the induction and maintenance of BIPN through activation of neuro-inflammatory processes [increases in pro-inflammatory substances (TNF, IL-1β, CCL2, CXCL1) and decreases in anti-inflammatory substances (IL-10, IL-4)]. Inhibiting the S1P-S1PR1 signaling pathway with FTY720, a functional antagonist of S1PR1, either during bortezomib treatment or when given 48 hours after the last dose of bortezomib, blocked the progression of BIPN throughout the observation period of 25 days. Fortunately, FTY720 is already FDA approved for the treatment of multiple sclerosis and could therefore be rapidly evaluated as an adjunct to bortezomib to block neuropathic pain. Importantly, FTY720 has well documented anti-cancer effects and inhibition of the S1P-S1PR1 axis is under investigation for anti-cancer treatments. Activation of TLR4 has been shown to be a key regulator of the release of pro-inflammatory cytokines. In our preliminary studies rats treated with bortezomib have increased TLR4 expression, pro-inflammatory cytokines, and chemokine/receptor transcription, and decreased anti-inflammatory cytokines. Inhibiting the S1P-S1PR1 axis with FTY720 leads to decreased TLR4 expression, pro-inflammatory cytokines, and chemokine/receptor transcription as well as increased anti-inflammatory cytokines. Overall these results show that inhibition of S1P-S1PR1 with FTY720 prevents the development of BIPN at least in part through modulation of neuro-inflammation in SC and implicate potential contribution of TLR4 in these effects. Additional studies are ongoing in support. Our results with FTY720 were extended to other S1PR1 antagonists such as NIBR14 strengthening our global hypothesis that targeting S1PR1 is an effective strategy to block neuropathic pain states.

Disclosures: **K. Stockstill:** None. **K. Janes:** None. **D. Salvemini:** None.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.08/I30

Topic: C.19. Drug Discovery and Development

Title: Preclinical profiling of a novel mglur8 positive allosteric modulator

Authors: ***M. COSDEN**[1], S. STACHEL[2], Y. ZHOU[1], J. J. RENGER[1], R. E. DROLET[1];
[1]Merck and Co., Inc., West Point, PA; [2]Merck, West Point, PA

Abstract: The metabotropic glutamate receptor 8 (mGluR8), is a presynaptic g-protein coupled receptor that inhibits neurotransmitter release through multiple presynaptic effector molecules. mGluR8 is enriched at several presynaptic sites within the CNS including the dorsal striatum, periaqueductal grey, amygdala, and nucleus tractus of the solitarius and as such remains an attractive target for multiple CNS disorders. However, the orthosteric binding site of metabotropic glutamate receptors (mGluR's), venus-fly-trap domains, are highly conserved among the eight mGluR subtypes and this hinders orthosteric agonist or antagonist development. Allosteric modulation however, has led to the development of selective compounds for several of the mGluR's. The usefulness and validation of mGluR8 as a target in the Neurosciences is hindered by the lack of selective and potent molecules that modulate the receptor. The data described in this presentation describe the preclinical *in vitro* and *in vivo* profiling of a novel mGluR8 positive allosteric modulator (PAM). The compound described here has *in vitro* activity in cell-based assays at sub 100nM concentrations, and has greater than 10-fold selectivity over other group III mGluR's, and no activity at group I or II mGluR's. The compound also has good selectivity against a broad panel of neuroscience-related protein targets and receptors with moderate activity against three un-related targets with potency values in the micromolar range. With respect to physicochemical properties, the compound is moderately soluble at pH7 and has reasonable plasma protein binding that predicts sufficient free plasma levels necessary to activate the receptor after peripheral dosing. The compound has good oral bioavailability and permeability and is not a PGP substrate that enables good CNS drug exposure. Consistent with this, the compound showed dose-dependent efficacy in a mouse formalin paw assay. Efficacy correlated well with free plasma drug exposure and was consistent with predicted *in vitro* potency values, indicative of mGluR8-dependent activity. Together, these data suggest this novel mGluR8 PAM molecule is a useful tool to evaluate mGluR8 activation as a potential target for multiple CNS disorders.

Disclosures: **M. Cosden:** A. Employment/Salary (full or part-time):; Merck. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Merck. **S. Stachel:** A. Employment/Salary (full or part-time):; Merck. **Y. Zhou:** A. Employment/Salary (full or part-time):; Merck. **J.J. Renger:** A. Employment/Salary (full or part-time):; Merck. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Merck. **R.E. Drolet:** A. Employment/Salary (full or part-time):; Merck. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Merck.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.09/I31

Topic: C.19. Drug Discovery and Development

Support: Pierre Fabre Laboratory

Title: F17475, a glutamatergic/NMDA receptor antagonist with a safer profile than ketamine: activity in a rat model of post-operative pain

Authors: *R. Y. DEPOORTERE[1], B. VACHER[1], A. AUCLAIR[1], J. C. MARTEL[1], P. HEUSLER[1], P. MOSER[1], M. GEORGY[2], T. CLERC[2];
[1]Lab. Pierre Fabre, Castres, France; [2]PFI, Lab. Pierre Fabre, Toulouse, France

Abstract: Glutamatergic/NMDA receptor antagonists, like ketamine, have benefited from a renewed interest recently, in particular for their anti-hyperalgesic and potential antidepressant properties. However, their propensity to produce side-effects (in particular dizziness and psychotomimetic symptoms) at doses close to or overlaping with those with therapeutic effect, has sparked an interest for ketamine-like compounds with a better safety margin. Here, we report the *in vitro* and *in vivo* activities of F17475 (3-amino-N,N-diethyl-1-phenylcyclobutanecarboxamide, patent WO2014086825-A1), using ketamine as a positive comparator. F17475 had slightly higher affinity than ketamine at rat NMDA receptors (Ki : 0.74 and 1.05 µM, respectively) and more potently blocked NMDA currents using cloned human NMDA receptors (IC50 : 0.52 and 0.74 µM, respectively). In addition, F17475 was more selective than ketamine against 120 targets. In the Brennan rat model of post-operative pain, using flinches as a marker of spontaneous pain (scored 24 h post-surgery), F17475 was as efficacious but at least twice as potent as ketamine, whether injected 1 h before or after surgery, or 1 h before scoring (MED: 1.25-2.5 and 5 mg/kg iv, for F17475 and ketamine, respectively) In addition, F17475 further potentiated the analgesic efficacy of morphine (0.63 to 2.5 mg/kg sc, 30 min pre-scoring), with a MED : 1.25 mg/kg iv when given 1 h pre-operatively, and a strong tendency at 2.5 mg/kg when given 1 h post-surgery. Ketamine showed a tendency to potentiate morphine at +1 h, but was ineffective at -1 h. In models of abuse liability (ketamine drug discrimination and locomotor activity in rats), F17475 was active at doses (MED : 10 and 25 mg/kg iv, respectively) above those active in the Brennan model, giving a safety ratio of 4 to 10. By contrast, ketamine produced effects at doses (MED : 1.25 and 10 mg/kg iv, respectively) below or just above analgesic ones, giving a safety ratio of 0.25 to 2. This advantageous safety profile was confirmed by the finding that F17475 produced no impact in an Irwin test up to 10

mg/kg iv, whereas ketamine produced signs from 0.63 mg/kg iv. These results suggest that F17475, a potent and efficacious glutamatergic/NMDA receptor antagonist, following iv administration, is at least as efficacious as, but more potent than ketamine in a rat model of post-operative pain. It is additionally endowed with a superior safety profile. These observations, combined with its opiate sparing potential, point towards the utility of F17475 as a substitute for ketamine, alone or in combination with opiates, for peri-operative pain relief, and prevention of chronification of pain.

Disclosures: **R.Y. Depoortere:** A. Employment/Salary (full or part-time):; Pierre Fabre Laboratory. **B. Vacher:** A. Employment/Salary (full or part-time):; Pierre Fabre Laboratory. **A. Auclair:** A. Employment/Salary (full or part-time):; Pierre Fabre Laboratory. **J.C. Martel:** A. Employment/Salary (full or part-time):; Pierre Fabre Laboratory. **P. Heusler:** A. Employment/Salary (full or part-time):; Pierre Fabre Laboratory. **P. Moser:** A. Employment/Salary (full or part-time):; Pierre Fabre Laboratory. **M. Georgy:** A. Employment/Salary (full or part-time):; Pierre Fabre Laboratory. **T. Clerc:** A. Employment/Salary (full or part-time):; Pierre Fabre Laboratory.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.10/I32

Topic: C.19. Drug Discovery and Development

Title: Selective enhancement of slow inactivation of Nav1.7 WT and pain-linked gain-of-function mutations

Authors: A. LAMPERT[1,2], A. O. O'REILLY[2], M. POHLER[2], J. MAJERCAK[3], *R. KLEIN[3]; [1]Inst. of Physiol., RWTH Aachen Univ. Hosp., Aachen, Germany; [2]Inst. of Physiol. and Pathophysiology, Friedrich-Alexander Univ. Erlangen-Nürnberg, Erlangen, Germany; [3]Merck Sharp and Dohme, West Point, PA

Abstract: Gain-of-function mutations of voltage-gated sodium channels are linked to inherited pain disorders and these channels are of pharmacological interest as targets for analgesic compounds. We investigated the effect of two novel compounds (A and B) and compare these results with the known sodium channel modulators mexiletine and carbamazepine. These compounds were tested on wild-type Nav1.7 and three different gain-of-function mutations associated with pain channelopathies. 1) L823R, which is linked to erythromelalgia, an inherited pain syndrome that is characterized by triggered attacks of intense burning pain, with onset

occurring in young adulthood. 2) I1461T, a paroxysmal extreme pain disorder (PEPD)-linked mutation, that induces excruciating pain attacks in children close to birth. 3) M1532I, a small fiber neuropathy (SFN) linked mutation, that induces gloves and stocking like symptoms of neuropathic pain in older adults. The voltage-dependence of activation of the PEPD and SFN mutants was shifted to more depolarized potentials by all tested compounds, whereas that of WT and the IEM mutation was unaffected. Fast inactivation was shifted to more hyperpolarized potentials for almost all Nav1.7 constructs and drugs tested. The most notable effect that compounds A and B had on WT Nav1.7 and all tested mutations was a potently-enhanced slow inactivation. The observed strong enhancement of slow inactivation at physiological resting membrane potentials suggests that these new compounds may represent promising new therapeutics for pain patients.

Disclosures: **A. Lampert:** 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.; Merck Sharp and Dohme. **A.O. O'Reilly:** 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.; Merck Sharp and Dohme. **M. Pohler:** 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.; Merck Sharp and Dohme. **J. Majercak:** A. Employment/Salary (full or part-time):; Merck Sharp and Dohme. **R. Klein:** A. Employment/Salary (full or part-time):; Merck Sharp and Dohme. 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.; Merck Sharp and Dohme.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.19. Drug Discovery and Development

Support: Alan Edwards Research Grant

Title: A meta-regression analysis of placebo response in clinical trials of neuropathic pain

Authors: *A. H. TUTTLE[1,5], S. TOHYAMA[1], J. KIMMELMAN[2], T. RAMSAY[6], G. J. BENNETT[3], P. SCHWEINHARDT[4], J. S. MOGIL[1,5];
[1]Dept. of Psychology, [2]Dept. of Biomed. Ethics, [3]Dept. of Anesthesia, [4]Dept. of Dent., McGill Univ., Montreal, QC, Canada; [5]Alan Edwards centre for research on pain, Montreal, QC, Canada; [6]Fac. of Med., Ottawa Hosp. Res. Inst., Ottawa, ON, Canada

Abstract: Increasingly, novel pharmacological targets for treating neuropathic pain are unable to pass randomized controlled drug trials, calling into question whether current drug study design may in part explain lack of demonstrable drug efficacy. In an effort to identify potential contributors to recent trial failures, we conducted a literature review of neuropathic pain placebo-controlled randomized clinical trials published between 1980 and 2014 using EMBASE, Medline, and Cochrane online databases. Specifically, a search for "neuropathic pain" and "drug," limited to randomized clinical trials, yielded a combined total of 1793 articles. We also included an additional 106 articles from a similar meta-analysis conducted by Finnerup et al. (2005), bringing our article total to 1899. First-pass inclusion criteria included: English language only, randomized controlled-trials, neuropathic pain conditions (except for migraine, trigeminal neuralgia, or orofacial pain), systemic drugs only, visual analogue scales or numeric rating scales, and parallel or cross-over trial design. After first-pass screening, 221 articles were included for additional analysis. After second-pass screening, a total of 80 articles were included in our final data set. Analysis of reported pain measures extracted from placebo and treatment groups yielded several striking correlations between study design and reported placebo-group pain scores. First, placebo ratings (reductions in pain measures) are becoming stronger over time. Furthermore, the increasing placebo response correlates with a significant decreasing trend in treatment advantage, indicating that increasing placebo response may negatively impact neuropathic pain trial outcome. However, our systematic review of the literature highlights several factors that might explain our correlations: Size of the study, number of treatment arms, and study duration/number of site visits appear to be significant correlative factors that predict an increase in placebo response. Finally, the magnitude of the placebo response appears to be limited to North American studies. Taken together, these findings are similar to those reported in both the depression and schizophrenia literature. While the magnitude of the placebo response appears to be increasing over time, causal mechanisms of these trends are still unknown.

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Poster

056. Pain, Headache and Migraine

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

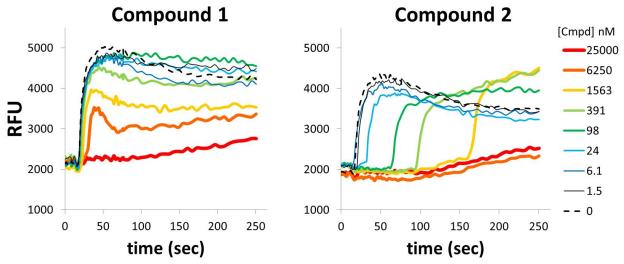
Program#/Poster#: 56.12/I34

Topic: C.19. Drug Discovery and Development

Title: Kinetic analysis of membrane potential dye response to NaV1.7 channel activation identifies antagonists with pharmacological selectivity against Nav1.5

Authors: ***M. F. FINLEY**[1], A. CONVERSO[2], M. CLEMENTS[2], C. DALEY[2], R. KRAUS[2], W. LEMAIRE[2], M. LAYTON[2], K. SOLLY[1], D. STAAS[2], J. WANG[2], M.-T. LAI[2], J. CASSADAY[1], T. KREAMER[1], X. LI[1];
[1]Automated Biotech., Merck, North Wales, PA; [2]Merck, West Point, PA

Abstract: The NaV1.7 voltage-gated sodium channel is a highly valued target for the treatment of neuropathic pain due to its expression in pain-sensing neurons and human genetic mutations in the gene encoding NaV1.7 resulting in either loss- (congenital analgesia) or gain- (paroxysmal extreme pain disorder) of-function pain phenotypes. We exploited existing technologies in a novel manner to identify selective antagonists of NaV1.7. A full-deck high-throughput screen was developed for both NaV1.7 and cardiac NaV1.5 channels using a cell-based membrane potential dye FLIPR assay. In assay development, known local anesthetic site inhibitors produced a decrease in maximal response; however, a subset of compounds exhibited a concentration-dependent delay in the onset of the response with little change in the peak of the response at any concentration. Therefore, two methods of analysis were employed for the screen: one to measure peak response and another to measure area under the curve (AUC) which would capture the delay-to-onset phenotype. Although a number of compounds were identified by a selective reduction in peak response in NaV1.7 relative to 1.5, only the AUC measurement and a subsequent refinement of this measurement were able to extract compounds with novel Nav1.7 pharmacological selectivity over Nav1.5 as confirmed in electrophysiology.



Disclosures: **M.F. Finley:** A. Employment/Salary (full or part-time):; Merck. **A. Converso:** A. Employment/Salary (full or part-time):; Merck. **M. Clements:** A. Employment/Salary (full or part-time):; Merck. **C. Daley:** A. Employment/Salary (full or part-time):; Merck. **R. Kraus:** A. Employment/Salary (full or part-time):; Merck. **W. Lemaire:** A. Employment/Salary (full or part-time):; Merck. **M. Layton:** A. Employment/Salary (full or part-time):; Merck. **K. Solly:** A. Employment/Salary (full or part-time):; Merck. **D. Staas:** A. Employment/Salary (full or part-time):; Merck. **J. Wang:** A. Employment/Salary (full or part-time):; Merck. **M. Lai:** A.

Employment/Salary (full or part-time):; Merck. **J. Cassaday:** A. Employment/Salary (full or part-time):; Merck. **T. Kreamer:** A. Employment/Salary (full or part-time):; Merck. **X. Li:** A. Employment/Salary (full or part-time):; Merck.

Poster

056. Pain, Headache and Migraine

Location: Hall A

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Program#/Poster#: 56.13/I35

Topic: C.19. Drug Discovery and Development

Support: NIH R01DA02328108

the State of Florida Executive Office of the Governor's Department of Economic Opportunity

Title: A novel functional assay for both μ-opioid receptor and nociception receptor

Authors: *J. WU, J. SCHOCH, M. WERGER, A. CIPPITELLI, L. TOLL;
Torrey Pines Inst. For Mol. Studies, Port Saint Lucie, FL

Abstract: Traditionally GTPgS binding and adenylyl cyclase (AC) activity inhibition are often used to measure μ-opioid receptor activation. We developed a novel assay in which intact live cells can be used to screen new ligands (agonist or antagonist) for both mu-opioid receptor and nociceptin receptor (NOP). Chinese hamster ovary cells were stably transfected with μ-opioid receptor and NOP receptors respectively. CHO cells expressing mu or NOP were seeded in 96-well plates, loaded with membrane potential-sensitive fluorescent dye for 60 minutes, and then treated with or without mu and NOP agonists. CHO-mu and CHO-NOP cells hyperpolarized upon the treatment of its own agonist, which is probably mediated by G protein-coupled inwardly-rectifying potassium channel or other certain potassium channel. Membrane potential change induced by CHO-MOR/NOP activation can only be detected only through membrane potential-sensitive fluorescent potassium dye but not through calcium dye. The resulting fluorescence changes could be detected in real time. The μ-opioid receptor agonist DAMGO has an EC50 of ~3.28nM in mu cells, and 100nM DAMGO-induced μ-opioid receptor activation can be inhibited by μ-opioid receptor antagonist naloxone in a dose-dependent manner, with an IC50 of is ~40nM. Similarly, NOP receptor agonist N/OFQ has an EC50 of ~14nM and activation of NOP receptors with 100nM N/OFQ can be inhibited by an NOP receptor antagonist in a dose-dependent manner with an IC50 of is ~30nM. This novel functional assay provides a simple, quick and safe method for real-time measurement of mu and NOP receptor activation or

inhibition through their action on native CHO cells. This method can greatly facilitate the drug discovery for opioid receptors.

Disclosures: **J. Wu:** None. **J. Schoch:** None. **M. Werger:** None. **A. Cippitelli:** None. **L. Toll:** None.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.14/I36

Topic: C.19. Drug Discovery and Development

Support: Rowan University Seed Funding Award

NIH Grant NS076517

Title: Antihyperalgesic effects of the α2 GABA-A and α3 GABA-A receptor positive allosteric modulator HZ-166 on pain-related stimulation and depression of behavior

Authors: *B. D. FISCHER[1], M. M. POE[2], J. M. COOK[2];
[1]Cooper Med. Sch. of Rowan Univ., Camden, NJ; [2]Univ. of Wisconsin, Milwaukee, WI

Abstract: GABAergic inhibition in the dorsal horn of the spinal cord is thought to contribute significantly to nociceptive processing. Drugs that enhance this inhibition produce antihyperalgesic effects in preclinical models, likely through GABA type A receptors that contain an α2 and/or α3 subunit (α2GABA-A receptors and α3GABA-A receptors, respectively). From a drug development perspective, it has been argued that the concurrent assessment of both pain-stimulated and pain-depressed behaviors may improve the translation of preclinical findings to the clinic. The purpose of the present study was to assess the antihyperalgesic effects of the α2GABA-A and α3GABA-A receptor positive allosteric modulator HZ-166 in a model of pain-stimulated behavior (withdrawal reflex from a mechanical stimulus) and a model of pain-depressed behavior (voluntary wheel running) in C57BL/6 mice. To assess pain-stimulated behavior, the yeast extract zymosan A (24 hr pretreatment; 0.06 mg/0.02 ml) was injected subcutaneously into the plantar surface of the footpad and mechanical sensitivity was assessed before and following HZ-166 administration (1.0 - 32 mg/kg, i.p.). Zymosan A increased sensitivity to the mechanical stimulus, resulting in a mean (± SEM) threshold of 3.8 ± 0.38 g. This increase in mechanical sensitivity was dose- and time-dependently reversed by HZ-166 and resulting in a peak threshold of 6.8 ± 0.26 g. In a separate group of mice, voluntary wheel

running was used as the dependent measure to assess pain-depressed behavior. Once stable wheel running was observed, complete Freund's adjuvant (CFA; 24 hr pretreatment; 0.01 ml) was injected bilaterally in to the plantar surface of both footpads. Injections of CFA reduced wheel running behavior to 1.0 ± 0.66 rotations/min during the onset of the dark cycle. Here, HZ-166 increased wheel running behavior to 8.1 ± 2.8 rotations/min during this same time period. These data suggest that systemic administration of a α2GABA-A and α3GABA-A receptor positive allosteric modulator produces an antihyperalgesic effect which may be observed in assays of both pain-stimulated and pain-depressed behavior. Together, these observations should provide a framework for studying GABA-A receptor pharmacology which in turn should help guide the development of improved therapeutic agents for the treatment of pain.

Disclosures: B.D. Fischer: None. **M.M. Poe:** None. **J.M. Cook:** None.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.15/I37

Topic: C.19. Drug Discovery and Development

Title: Electrophysiology studies and homology modeling to increase the selectivity of NaV1.7 inhibitors

Authors: R. KLEIN, A. ROECKER, *M. CLEMENTS, C. DALEY, D. WANG, M. LAYTON, V. SANTARELLI, J. MAJERCAK, R. KRAUS, A. HOUGHTON;
Merck, West Point, PA

Abstract: NaV1.7 is a voltage-gated sodium channel genetically validated to play a critical role in nociception and pain. Local anesthetics (LAs) are effective at reducing pain but lack subtype selectivity, resulting in safety risks. Our team has identified small molecule NaV blockers with selectivity between NaV1.7 and NaV1.5 (cardiac isoform) and identified their binding site by manual patch clamp using an innovative chimeric channel approach. This approach, using a library of NaV1.7/1.5 chimeric constructs, led to the identification of a novel binding pocket in the less-conserved DIV,S2S3 voltage sensor region of the sodium channel alpha-subunit. This site is distinct from both the highly conserved LA binding site (DIV,S6 pore region) and other known toxin inhibition sites. From these efforts, a homology model was constructed to inspire design elements that led to the development of novel and potent aryl-sulfonamide compounds, offering the potential for highly selective NaV1.7 inhibitors. Furthermore, these mutagenesis and homology studies identified key point residues, Y1537 (DIV, S2), W1538 (D1V,S2), and A1585

(DIV,S3), responsible for aryl-sulfonamide binding and selectivity over NaV1.5. This approach continues to inspire target design and aids in rationalization of selectivity over NaV isoforms.

Disclosures: **R. Klein:** A. Employment/Salary (full or part-time):; Merck Sharp and Dohme. **A. Roecker:** A. Employment/Salary (full or part-time):; Merck Sharp and Dohme. **M. Clements:** A. Employment/Salary (full or part-time):; Merck. **C. Daley:** A. Employment/Salary (full or part-time):; Merck. **D. Wang:** A. Employment/Salary (full or part-time):; Merck. **M. Layton:** A. Employment/Salary (full or part-time):; Merck. **V. Santarelli:** A. Employment/Salary (full or part-time):; Merck. **J. Majercak:** A. Employment/Salary (full or part-time):; Merck. **R. Kraus:** A. Employment/Salary (full or part-time):; Merck. **A. Houghton:** A. Employment/Salary (full or part-time):; Merck.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.16/I38

Topic: C.19. Drug Discovery and Development

Support: NIH Grant DA031243

UICentre for Drug Discovery

Department of Psychiatry UIC

Title: Stimulation of soluble guanylate cyclase triggers migraine-associated pain

Authors: ***A. A. PRADHAN**[1], A. F. TIPTON[1], R. GHANDI[2], L. SEGURA[1], A. ACHARYA[1], G. THATCHER[2];
[1]Psychiatry, [2]Medicinal Chem. & Pharmacognosy, UIC, Chicago, IL

Abstract: Migraine is a complex brain disorder that affects hundreds of millions of individuals worldwide. Available treatments are only effective in a limited number of patients; and there is a desperate need for novel drug therapies. Nitroglycerin (NTG) is a known migraine trigger, and produces migraine-associated hyperalgesia in mice. How NTG induces migraine is unclear, as it activates the soluble guanylate cyclase pathway, but also produces radical oxygen species leading to oxidative stress. The aim of this study was to determine the specific contribution of the soluble guanylate cyclase (sGC) pathway to migraine-associated pain. C57Bl6/J mice were treated acutely and chronically with either vehicle or the sGC stimulator VL-102. Basal and post-treatment mechanical responses were determined using von Frey hair stimulation. VL-102

produced acute hyperalgesia in a dose-dependent manner. Chronic administration of VL-102 produced both acute and basal hypersensitivity. VL-102-induced hyperalgesia was blocked by the anti-migraine medications sumatriptan and topiramate. These results are similar to the migraine-related pain induced by NTG. Stimulation of guanylate cyclase mimics the effects of NTG-induced pain, and appears to be migraine-associated. These results show that the effects of NTG on migraine are due to direct activation of the nitric oxide pathway. Furthermore, this work indicates that soluble guanylate cyclase inhibitors may be a novel therapeutic target for the treatment of migraine.

Disclosures: **A.A. Pradhan:** None. **A.F. Tipton:** None. **R. Ghandi:** None. **L. Segura:** None. **A. Acharya:** None. **G. Thatcher:** None.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.17/I39

Topic: C.19. Drug Discovery and Development

Title: Inhibition of electrical-field stimulation-evoked signals in cultured mouse DRG neurons by sodium channel inhibitors is highly predictive of their ability to increase the threshold to action potential firing as measured by current clamp

Authors: ***C. DALEY II**[1], J. WANG[1], I. GREGAN[1], A. HOUGHTON[1], M. KARLSSON[2], S. LARDELL[2], C. LINDWALL-BLOM[2], P. KARILA[2];
[1]Ion Channel Pharmacol., Merck, West Point, PA; [2]Cellectricon, Molndal, Sweden

Abstract: Manual patch clamp recordings performed in the current clamp configuration, using cultured dorsal root ganglion (DRG) neurons, afford unique insight into the excitability of native tissue. Patch clamp using this native tissue allows one to examine the pharmacological effects of modulators of cellular excitability in a physiologically relevant configuration. In this way, electrical current is applied to the patched neuron at various frequencies and current amplitudes in order to evoke a depolarization of the membrane which is initiated by the opening of voltage-gated sodium channels (VGSCs). In these native tissue, the opening of VGSCs initiates the start of membrane depolarization from the resting potential, subsequently resulting in the opening of voltage gated calcium channels (VGCCs) and action potential firing. While the current clamp technique provides unparalleled insight into the excitability of native tissue, it is extremely low-throughput, requiring high-quality native tissue sources, delicate tissue digestion and culture conditions, and highly experienced electrophysiologists trained in patch clamp recording.

Alternatively, by utilizing an electrical field stimulation (EFS) electrode head to induce membrane depolarization in DRG cultures, in combination with a calcium-sensitive fluorescent dye and rapid image acquisition, the action potential can be indirectly monitored in excitable cells. The EFS technique therefore provides a dramatically higher-throughput means of quantifying the excitability of native tissues. Here we describe the use of an EFS system custom-built by Cellectricon, in the development of an assay using mouse DRG neurons, which is sensitive to sodium channel inhibitors. We found an excellent correlation between sodium channel blockade measured by EFS and by conventional current clamp. Prototypical sodium channel blockers tetrodotoxin (TTX), and tetracaine inhibited the EFS signal, as did internally developed, subtype-selective small molecule inhibitors of NaV1.7. This correlation strongly suggests that this EFS platform can be used to screen for inhibitors of VGSC-driven action potential firing in a high-throughput manner, enabling triage of compounds likely to modulate the excitability of native tissue earlier on in the drug development cycle.

Disclosures: **C. Daley II:** A. Employment/Salary (full or part-time):; Merck. **J. Wang:** A. Employment/Salary (full or part-time):; Merck. **I. Gregan:** A. Employment/Salary (full or part-time):; Merck. **A. Houghton:** A. Employment/Salary (full or part-time):; Merck. **M. Karlsson:** A. Employment/Salary (full or part-time):; Cellectricon. **S. Lardell:** A. Employment/Salary (full or part-time):; Cellectricon. **C. Lindwall-Blom:** A. Employment/Salary (full or part-time):; Cellectricon. **P. Karila:** A. Employment/Salary (full or part-time):; Cellectricon.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.18/I40

Topic: C.19. Drug Discovery and Development

Support: NIH DA031243

UIC Department of Psychiatry

UICentre

Title: Inhibition of soluble guanylate cyclase alleviates migraine-associated pain

Authors: ***A. F. TIPTON**[1], R. GANDHI[2], Y. WANG[2], G. THATCHER[2], A. A. PRADHAN[1]; [1]Psychiatry, Univ. of Illinois At Chicago, Chicago, IL; [2]Medicinal Chem. and Pharmacognosy,, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Migraine is an extraordinarily common brain disorder for which therapeutic options continue to be limited. The nitric oxide pathway has been heavily implicated in migraine, and the nitric oxide donor nitroglycerin (NTG) has been shown to reliably trigger migraine in humans. NTG stimulates soluble guanylate cyclase (sGC), the main NO receptor in the body, which increases production of cGMP. The guanylate cyclase pathway is of particular relevance to migraine as upregulation of cGMP by NTG or sildenafil (a phosphodiesterase 5 inhibitor) produces headache but no other type of pain. Previously we have shown that stimulating sGC produces a migraine-associated pain in mice, which was reversed by prototypic anti-migraine medications. The aim of this study is to determine the effectiveness of sGC inhibitors as novel anti-migraine therapies. C57bl6/J mice were treated acutely and chronically with either vehicle or RG2-12 prior to administration of NTG, vehicle, or the sGC stimulator VL-102. Basal and post-treatment mechanical thresholds were determined using von Frey hair stimulation. RG2-12 reversed acute hyperalgesia and basal hypersensitivity induced by both VL-102 and NTG. Acute treatment with RG2-12 also alleviated established hyperalgesia produced by chronic administration of NTG or VL-102. These results indicate that inhibition of soluble guanylate cyclase alleviates migraine-associated hyperalgesia in a mouse model of acute and chronic migraine. These data indicate that soluble guanylate cyclase inhibitors could be a promising therapeutic target for the treatment of migraine. This work was supported by NIH DA031243, the Department of Psychiatry UIC, and the UICentre

Disclosures: A.F. Tipton: None. **R. Gandhi:** None. **Y. Wang:** None. **G. Thatcher:** None. **A.A. Pradhan:** None.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.19/I41

Topic: C.19. Drug Discovery and Development

Title: Effect of SUVN-H1106036, a CB2 receptor agonist, on spinal wide dynamic range neuronal activity in neuropathic rats

Authors: V. GOURA, *A. K. SHINDE, A. VUYYURU, R. KALLEPALLI, P. JAYARAJAN, R. ABRAHAM, S. DARIPELLI, V. KAMUJU, G. BHYRAPUNENI, V. BHATTA, K. KANDUKURI;
Suven Life Sci., HYDERABAD, India

Abstract: A selective canabinoid 2 receptor (CB2) agonist SUVN-H1106036 showed anti-nociceptive effects in rat models of pain. We investigated the effect of SUVN-H1106036 on wide dynamic range dorsal horn neuronal firing in an animal model of nerve injury. Chronic constricted sciatic nerve injury model of neuropathic rat was selected after 2-3 weeks of the nerve ligation. Selected rats were used for investigation of neuronal excitability in dorsal horn region of the spinal cord using *in vivo* electrophysiology techniques. Microdialysis was carried out in dorsal horn in order to evaluate the changes in monoamine levels. Peripheral nerve injury is associated with increased excitability of sensory neurons and decreased monoaminergic transmission. SUVN-H1106036 and Gabapentin attenuated spontaneous and mechanically-evoked neuronal firing of spinal wide dynamic range neurons. In spinal microdialysis SUVN-H1106036 elevated the levels of monoamines providing the neurochemical basis for the anti-nociceptive activity of the test compound.

Disclosures: V. Goura: A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **A.K. Shinde:** 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. Kallepalli:** 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. Abraham:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **S. Daripelli:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **V. Kamuju:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **G. Bhyrapuneni:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **V. Bhatta:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD. **K. Kandukuri:** A. Employment/Salary (full or part-time):; Suven Life Sciences LTD.

Poster

056. Pain, Headache and Migraine

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 56.20/I42

Topic: D.08. Pain

Support: R01NS/069572

Title: Dural sensory innervation expresses unique opioid and adrenergic receptor subtypes: Implications for migraine pathology and treatment

Authors: F. L. RICE[1], J. R. BOURGEOIS[2], J. Y. XIE[3], C. M. KOPRUZINSKI[3], N. EYDE[3], F. PORRECA[3], *P. J. ALBRECHT[2];

[1]Integrated Tissue Dynamics, LLC, Rensselaer, NY; [2]Cntr Neuropharmacol/Neurosci, Albany Med. Coll, Albany, NY; [3]Dept. of Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: Effective prevention and treatment of migraine headaches remains a major clinical challenge. A high priority therapeutic target has become the potent vasodilator Calcitonin Gene Related Peptide (CGRP), which is increased in jugular vein blood of people with migraines. A postulated source of the increased CGRP is hyperactivity of trigeminal sensory innervation to meningeal arteries of the dura mater, with resultant neurogenic inflammation (excessive vasodilatation, plasma extravasation, and mast cell infiltration) as the underlying etiology of pain. However, despite many studies, a comprehensive assessment of the multi-molecular characteristics of dural innervation remains lacking. Here, we discriminate among molecular varieties and distributions of sensory and autonomic innervation of the dura to provide new high-priority target identification and evidence for rational development of novel therapeutic strategies. We used the highly predictable patterns of meningeal arteries in adult male rats to collect identical sectors of 4% paraformaldehyde fixed dura. The repeatable whole-mount samples enabled compiling of double and triple immunolabel combinations designed to profile innervation based on functionally-implicated neurochemical characteristics. Our work emphasizes the extensive and diffuse innervation of the dura between the meningeal arterial arbors among a dense capillary meshwork. Utilizing the pan-neuronal marker PGP9.5 as the baseline for total innervation immunolabeling, we identified noradrenergic sympathetic innervation co-expressing neuropeptide Y (NPY), Tyrosine Hydroxylase (TH), and Dopamine beta-Hydroxylase (DβH), at least two type of likely Aδ fibers co-expressing 200kD neurofilament protein (NF) and myelin basic protein (MBP), and several varieties of C-fibers (NF- and MBP-negative) that mostly express CGRP. Importantly, intimate intertwining of individual sympathetic fibers and sensory C-fibers expressing CGRP and that also co-expresses an α-adrenergic receptor were observed, suggesting a sympathetic regulation of the sensory fiber activity. Furthermore, we observed that nearly all dural peptidergic (CGRP-positive) C-fibers bind the isolectin IB4, and coexpress the δ-opioid receptor (δOR), while largely lacking μOR expression. This contrasts with cutaneous peptidergic C-fibers of which most lack IB4 binding, and express μOR but not δOR. These important properties of dural sensory innervation provide the basis for novel migraine treatment strategies.

Disclosures: **F.L. Rice:** None. **J.R. Bourgeois:** None. **J.Y. Xie:** None. **C.M. Kopruzinski:** None. **N. Eyde:** None. **F. Porreca:** None. **P.J. Albrecht:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Integrated Tissue Dynamics, LLC.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 57.01/I43

Topic: D.02. Auditory System

Support: R01DC04682

Title: *In vivo* imaging of mouse auditory cortex during associative fear learning

Authors: *S. N. GILLET[1], H. K. KATO[2], M. A. JUSTEN[2], J. S. ISAACSON[2];
[1]Ctr. for Neural Circuits and Behavior, [2]UC San Diego, La Jolla, CA

Abstract: Although previous work has suggested that auditory fear conditioning causes a rapid increase in cortical representations to conditioned tones, how sensory representations are modulated across cortical layers is unclear. To address this question we developed a strategy for studying auditory fear learning in head-fixed mice. This approach allows us to use chronic two-photon imaging and GCaMP6 to examine the activity of identified cell populations throughout all phases of auditory fear learning (conditioning, memory retrieval, and extinction). We first used an optogenetic approach to determine whether auditory cortex is required for discriminative memory retrieval. VGAT-ChR2 mice and LED illumination were used to rapidly silence firing activity across all layers of auditory cortex. Head-fixed mice were conditioned in a discriminative fear learning task in which the suppression of licking behavior indicated a fear response. Mice were conditioned with two FM tones differing by one octave: one paired with a mild tail shock (CS+), and the other unpaired (CS-). Fear memory retrieval was tested one day after conditioning. On control trials, mice showed a robust fear response to the CS+, but not the CS-. In contrast, optogenetic inactivation of the auditory cortex contralateral to the sound stimuli on interleaved trials abolished the animals' ability to discriminate between the CS+ and CS- tones. In control experiments, discriminative responses were intact when the ipsilateral cortex was inactivated during memory retrieval. These results indicate that auditory fear conditioning is effective in head-fixed mice and that the auditory cortex is required for the correct discrimination of CS tones. We next examined whether discriminative fear conditioning modulates cortical sensory representations. We performed chronic, *in vivo* two-photon imaging of layer 5 pyramidal cells and imaged responses of the same populations of neurons to the CS+ and CS- before and after tone/shock conditioning. Preliminary experiments suggest that layer 5 sensory representations of the two tones become more dissimilar after conditioning. These data suggest that auditory cortex contributes to discriminative learning via an increase in contrast between behaviorally relevant and irrelevant tones.

Disclosures: **S.N. Gillet:** None. **H.K. Kato:** None. **M.A. Justen:** None. **J.S. Isaacson:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

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Program#/Poster#: 57.02/I44

Topic: D.02. Auditory System

Support: NIH RO1 DC04682

JSPS Postdoctoral Fellow for Research Abroad

Title: Experience-dependent modulation of sound representations in mouse primary auditory cortex

Authors: *H. K. KATO, S. N. GILLET, J. S. ISAACSON;
UCSD Sch. of Med., La Jolla, CA

Abstract: Acoustic experience during early development modulates the maturation of sound representations in the auditory system. During brief critical periods in rodents, repeated passive exposure to a tone results in enlarged cortical representations of that tone. In contrast, the adult nervous system is largely refractory to this treatment. Here we use chronic, two-photon Ca2+ imaging to reinvestigate the effects of simple passive experience on the responses of the same populations of neurons in primary auditory cortex (A1). We find a marked reduction in excitatory responses of layer 2/3 pyramidal neurons to tones experienced repeatedly across five days. This “habituation” is accompanied by an increase in the fraction of neurons with inhibitory responses to the experienced tone. This increased inhibition was not observed in thalamocortical recipient layer 4, suggesting a cortical source of layer 2/3 plasticity. Furthermore, cell type-specific Ca2+ imaging revealed that habituation enhanced the activity of somatostatin-expressing, but not parvalbumin-expressing interneurons. Together, our results demonstrate the neuronal correlates of habituation in A1 and suggest a role for specific local inhibitory circuits in this modulation. Currently, we are studying how positive associative learning changes sound representations.

Disclosures: **H.K. Kato:** None. **S.N. Gillet:** None. **J.S. Isaacson:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.02. Auditory System

Support: NIH Grant DC013826

Helen Hay Whitney Foundation

Title: Neural coding of self-generated sounds in mouse auditory cortex

Authors: *D. M. SCHNEIDER, R. MOONEY;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Sounds generated by our own movements (reafferent sounds) often co-occur with sounds emanating from the environment (exafferent sounds), and distinguishing between these two sound classes is critical to many forms of auditory-guided motor learning, such as speech and musicianship. To distinguish between environmental and self-generated sounds, the brain may take advantage of the fact that self-generated sounds can be anticipated, since they are time-locked to our movements and their acoustic features are often predictable. One strategy for exploiting this predictability is through copies of motor-related commands, termed corollary discharge, that are sent to the auditory system, where they may suppress or otherwise modulate predictable self-generated sounds. A corollary discharge circuit connecting the motor cortex to the auditory cortex in the mouse is active during movement and suppresses auditory cortical pyramidal neurons, providing a potential substrate for such a mechanism. However, it is not known whether copies of motor-related signals acting at the level of the auditory cortex specifically modulate self-generated sounds. To address this question, we developed a novel acoustic virtual reality paradigm in which we can precisely control and manipulate the timing, spectral features and predictability of the sounds produced by a mouse's movements. Mice ran on a quiet, non-motorized treadmill, the rotational velocity of which was monitored in real time, and brief tone pips of a fixed pitch were presented through a speaker at a rate proportional to the treadmill's velocity. Over several days of acclimation, mice ran the equivalent of many hundreds of meters and heard several thousand reafferent tone pips. After acclimation, we made extracellular recordings from multiple auditory cortical neurons while mice ran and rested on the treadmill and listened to expected reafferent sounds as well as unexpected "probe" sounds that deviated from the anticipated pitch. Preliminary experiments indicate that for the majority of auditory cortical neurons, sound-evoked responses are suppressed during movement, but that the sign and degree of movement-related modulation is heterogeneous and a subset of neurons have enhanced responses during movement. Ongoing experiments aim to determine the degree to which movement-related modulation is specific to predictable self-generated sounds and to identify circuits through which neural responses to self-generated sounds may be selectively modulated.

Disclosures: D.M. Schneider: None. **R. Mooney:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

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Program#/Poster#: 57.04/I46

Topic: D.02. Auditory System

Support: Akdeniz University Scientific Research Projects Management Committee (Project Number: 2009.03.0122.001)

Title: The effects of pre and post-natal exposure to extremely low frequency electric fields on mismatch negativity component of the auditory event-related potentials

Authors: *P. A. YARGICOGLU[1], D. AKPINAR[2], D. KANTAR GOK[2], M. ASLAN[3], S. OZEN[4];
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Abstract: Most public exposure to extremely low frequency electric fields (ELF-EFs) comes from electrical appliances, alternating current (AC) transmission and distribution lines. There was no study examining developmental effects of 50 Hz Electric fields (EFs) on Mismatch Negativity (MMN) recorded from rats. Therefore, our study aimed to investigate MMN, apoptosis and oxidative brain damage in rats exposed to pre and/or postnatal 50 Hz EF at 12 kV/m intensity for 1 hour per day. Forty Wistar male rats were divided into four groups; Control group (C), prenatal group were exposed to EF during pregnancy (Pr), post-natal group were exposed to EF after pregnancy for three months (Po), pre+postnatal group were exposed to EF during pregnancy and continued to be exposed to EF during three months (PP). Pregnant rats of Pr and PP groups were exposed to 50 Hz EF (12 kV/m; 1 h/day) while those of C and Po groups were kept under the same experimental conditions without being exposed to any EF during pregnancy. Following parturition, rats of PP and Po groups were exposed to EF whereas rats of C and Pr groups were kept under the same experimental conditions without being exposed to any EF during 90 days. Post natal day 90, MMN were recorded in urethane-anesthetized rats by electrodes positioned stereotaxically to the surface of the dura. After recordings, brain tissues were removed for histological and biochemical analysis. The MMN amplitude was higher to deviant tones than to standard tones. It was decreased in all experimental groups compared with the C group. 4-hydroxy-2-nonenal (4-HNE) levels were significantly increased in the Po group with respect to the C group whereas they were significantly decreased in the PP group compared

with Pr and Po groups. Protein carbonyl levels were significantly decreased in the PP group compared with C, Pr and Po groups. In conclusion, EF decreased MMN amplitudes possibly induced by lipid peroxidation.

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Poster

057. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.02. Auditory System

Support: Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS), Japan Agency for Medical Research and development (AMED)

Brain Science Project of the Center for Novel Science Initiatives (CNSI), National Institutes of Natural Sciences (NINS) (BS261006)

Title: Magnitude of stimulus deviance influences on mismatch activity in common marmosets

Authors: *M. KOMATSU, K. TAKAURA, N. FUJII;
RIKEN Brain Sci. Inst., Saitama, Japan

Abstract: Mismatch negativity (MMN) is a component of event-related potentials evoked by violations of the regularity in sensory stimulus-sequences in human. Recently, MMN has received attention as a clinical and translatable biomarker of psychiatric disorders such as schizophrenia to develop animal models of these psychiatric disorders. In this study, we investigated MMN in common marmosets, which is an important non-human primate model with genetic manipulability. We recorded the electrocorticograms (ECoGs) from two common marmosets with epidurally implanted electrodes covering a wide range of cortical regions. The 28- or 32- channel ECoG array was epidurally implanted on the left hemisphere of each marmoset. ECoG recordings were conducted in passive listening condition with a roving oddball paradigm. Repetitive tone-sequences with 20 types of frequency (250 - 6727 Hz with an interval of 1/4 octaves) are randomly presented. We considered the last tones of sequences as standard, and the first tones of sequences as deviants. First, we calculated ERPs for standards and deviant stimulus, respectively. Then, difference wave is obtained by subtracting the deviant stimulus ERP from the standard stimulus ERP. We observed significant negative and positive components

of the difference wave, which have a peak between 50-250 ms after the onset of the deviants, in the temporal, frontal, and parietal areas of marmosets' brain. Furthermore, we investigated the influence of the difference between standard and deviant frequencies on the amplitude of those significant components. Some of those components in the temporal and frontal areas revealed a correlation between its amplitude and the magnitude of stimulus deviance. These results comparable with MMN in human and macaque monkey, and mismatch activity in common marmoset seems to provide a tool to understand the neural basis of MMN.

Disclosures: **M. Komatsu:** None. **K. Takaura:** None. **N. Fujii:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 57.06/I48

Topic: D.02. Auditory System

Support: Brain Science Project of CNSI at NINS, BS261006

Title: Priming effect of a stimulus-repetition: Repetitive presentation of a stimulus facilitates the cortical response to the other stimuli subsequent to the repetition

Authors: *K. TAKAURA, M. KOMATSU, N. FUJII;
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Abstract: An event-related potential called the mismatch-negativity (MMN) is a possible neural signature of the ability to detect changes in the sensory signal-flow. MMN has been assessed by subtraction of the neural response to the repeatedly presented stimulus (Standard) from the response to the deviant stimuli (Deviant) subsequent to the repetition, which leads to a debate whether MMN can be solely explained by the stimulus-specific adaptation (SSA), a phenomenon of the neural response attenuation with the repetitive presentation of a stimulus. An early literature (Javitt et al., 1996) proposed an additional neuronal mechanism, arguing that the repetition of one stimulus leads to the decrease of the inhibition-level of the neurons not sensitive to the repeated one, but this idea has not received much empirical supports. To address this issue, we examined the effect of the repetition-number on the cortical responses to Standard, Deviant, and the mismatch-activity (MMA), respectively. If the above idea is correct, the population-response to Deviant could be enlarged by the increase in the number of the preceding repetition. We recorded the electrocorticogram from a wide range of the cortical regions in two macaque monkeys passively exposed to the auditory roving oddball-sequence. Trains of 3, 5 or 11 same

tone was pseudo-randomly presented without inter train pauses. We used 20 kinds of tones different in pitch, and the stimulus onset asynchrony was 503 ms. We took the last tone in each train of n-tones (n = 3, 5, or 11) and the first tone of the subsequent train as a pair of Standard and Deviant for n-repetition. As the number of repetition increased, the significantly larger response to Deviant than Standard, i.e. MMA, occurred in more widely distributed cortical regions involving the temporal, frontal and parietal cortices, and MMA in each electrode became larger. Surprisingly, the increment of MMA as a function of the repetition-number was mainly due to the augmentation of the response to Deviant. The attenuation of the response to Standard was less pronounced. We also observed that Deviant following 11-repetition evoked the auditory response in a subset of the electrodes on the frontal cortex and anterior part of the temporal cortex that were not responsive to the stimuli presented with less or without preceding repetition. These results cannot be explained by SSA, and indicate that the repetitive presentation of one-stimulus facilitates the response of the brain to the other stimuli following the repetition, which seems compatible with the proposal by Javitt et al (1996). MMN would relay on not only SSA, but also on the facilitative effect of the stimulus-repetition.

Disclosures: **K. Takaura:** None. **M. Komatsu:** None. **N. Fujii:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.02. Auditory System

Support: NIDCD R01DC014479

NIDCD R93DC013660

Klingenstein Award in Neurscience

Human Frontier in Science Young Investigator Award RGY0073/2014

Burroughs Wellcome Career Award at Scientific Interface

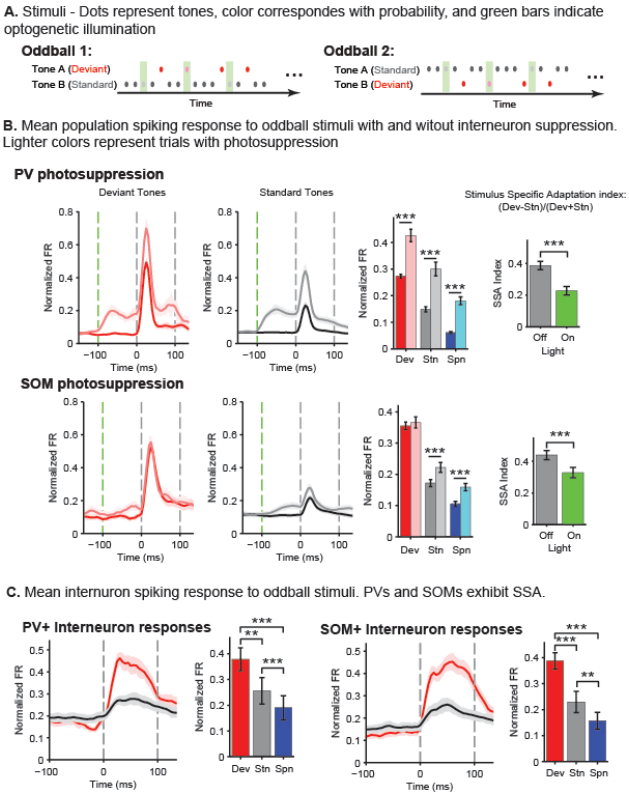
NIH NIMH T32MH017168

NIH NEI T32EY007035

Title: Multiple mechanisms for stimulus-specific adaptation in the primary auditory cortex

Authors: *R. G. NATAN[1], J. J. BRIGUGLIO[1], L. MWILAMBWE-TSHILOBO[1], E. M. GOLDBERG[3,2], M. N. GEFFEN[1];
[1]Dept. of Otorhinolaryngology, [2]Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA; [3]Div. of Neurol., The Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Adaptation to stimulus context is a ubiquitous property of cortical neurons and is thought to enhance efficiency of sensory coding. Yet the specific neuronal circuits that facilitate cortical adaptation remain unknown. In the primary auditory cortex (A1), the vast majority of neurons exhibit stimulus-specific adaptation (SSA), responding weakly to frequently repeated tones and strongly to rare tones in oddball stimuli. This form of history-dependent adaptation may increase cortical sensitivity to rare sounds. Here, we identify three distinct components shaping cortical SSA. The current source density sink amplitude profile across cortical layers in response to common and rare tones revealed that thalamo-cortical inputs to A1 exhibit SSA, albeit at lower levels than spiking responses. Furthermore, we found that two types of inhibitory interneurons contribute to SSA in a complementary fashion. Optogenetic suppression of parvalbumin-positive interneurons (PVs) led to an equal increase in the putative excitatory neuron firing rates to both common and rare tones (C). Suppression of somatostatin-positive interneurons (SOMs) led to an increase in neuronal responses to frequent, but not to rare tones. While the inhibitory effect of SOMs on excitatory neurons increased with successive tone repeats, PVs provided constant inhibition throughout the stimulus sequence. The effects of PVs and SOMs differed across cortical layers, consistent with their distribution across layers. Remarkably, both PVs and SOMs exhibited stimulus-specific adaptation. We constructed a firing rate model of a coupled excitatory-inhibitory neuronal population. Our simulation revealed how inhibitory neurons, despite exhibiting firing rate adaption themselves, increase adaptation in excitatory neurons. Taken together, our results demonstrate that SSA in the auditory cortex is a product of multiple adaptation mechanisms.



Disclosures: **R.G. Natan:** None. **J.J. Briguglio:** None. **L. Mwilambwe-Tshilobo:** None. **E.M. Goldberg:** None. **M.N. Geffen:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

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Program#/Poster#: 57.08/J2

Topic: D.02. Auditory System

Support: Studientstiftung des Deutschen Volkes

Quebec's National Research Fund for Nature and Technologies [181120]

Graduiertenkolleg [GRK 1589]

DFG 1196/5-1

DFG Cluster of Excellence [EXC 1077/1]

Title: On reward prediction errors and sensory representations: a role for dopamine in refining perception

Authors: *R. HOLCA-LAMARRE[1,2], K. OBERMAYER[1,2], J. LUECKE[3,1];
[1]Technische Univ. Berlin, Berlin, Germany; [2]Bernstein Ctr. for Computat. Neurosci., Berlin, Germany; [3]Dept. of Med. Physics and Acoustics, Univ. Oldenburg, Oldenburg, Germany

Abstract: The ventral tegmental area (VTA) contains dopamine releasing neurons whose activity reflects reward prediction errors. Activity in the VTA has a potent effect on cortical sensory representations: pairing stimulation of the VTA with presentation of an auditory tone, for instance, increases the representation area of this tone in the primary auditory cortex. It is unclear why a signal related to reward prediction errors should have such an effect on cortical representations. Here, we use a neural network model to examine this question. We extend a model of synaptic plasticity and representational learning to reproduce the effects of VTA activation on the network's representation: in the model, as in animals, pairing stimulus presentation with VTA activation shifts the synaptic weights of neurons towards the paired stimulus. The network is subjected to a classification task and is rewarded for taking correct classification decisions. Additionally, at each trial, the network makes a prediction about the reward it expects to receive. The difference between the predicted and received reward makes up a reward prediction error; this error activates the VTA. We perform parameter exploration to determine the optimal VTA activation depending on the value of the reward prediction error. We find that the VTA activation profile that is optimal with respect to classification performance in the model matches the activation profile observed in animals. During training, VTA activation refines synaptic weights with respect to the classification task and significantly improves the network's performance on the task. Our model therefore provides an explanation as to why reward prediction error signals affect sensory representations in animals.

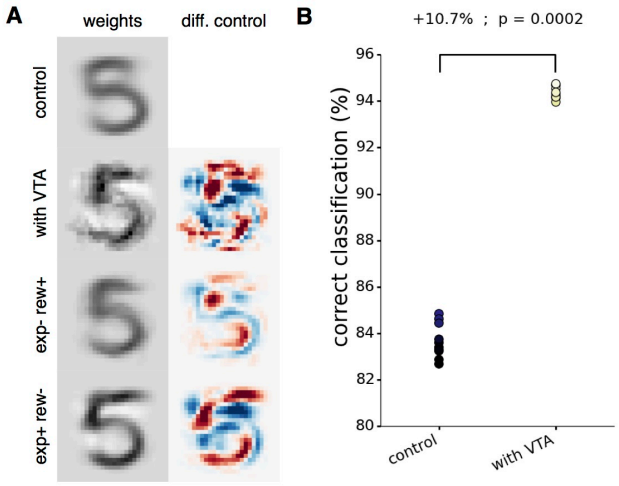

*Figure 1: Modelling the effects of VTA activation in the network refines synaptic weights **(A)** and leads to large improvements in classification performances **(B)**.*

Disclosures: **R. Holca-Lamarre:** None. **K. Obermayer:** None. **J. Luecke:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.02. Auditory System

Support: NIMH Grant K23MH101637-01A1

Title: Neural evidence for non-reward prediction errors in the sensory domain

Authors: ***G. HORGA**[1], E. JUNG[2];
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Abstract: Predictive-coding models suggest that the brain uses internal, predictive models of the external world to anticipate forthcoming stimuli and minimize coding of redundant information. A wealth of evidence indicates that similar predictive codes govern reward processing, but little is known about whether predictive coding also supports sensory processing. Here, using an axiomatic approach derived from neuroeconomics we formally tested whether healthy human participants signal (non-reward) sensory prediction errors. Participants completed a probabilistic speech discrimination task that explicitly manipulated expectations for hearing speech or non-speech vocal sounds on each trial (probabilities of speech vs. non-speech: 0, 0.25, 0.5, 0.75, 1). Functional magnetic resonance imaging (fMRI) was acquired while participants performed the task to determine whether neural signals at the presentation of speech or non-speech auditory stimuli conformed to sensory prediction errors. Behaviorally, participants discriminated stimuli more accurately and faster on trials that presented more certain information (0/1>0.25/0.75>0.5), indicating that they incorporated probabilistic information into the decision process. Post-task pleasantness ratings indicated that speech and non-speech stimuli were equally pleasant. Analysis of fMRI data revealed that blood-oxygen-level-dependent signals in voice-selective regions of temporal cortex and ventral striatum tracked non-reward, signed prediction errors. These findings suggest that predictive coding is a general coding scheme used by brain systems outside the reward domain.

Disclosures: **G. Horga:** None. **E. Jung:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 57.10/J4

Topic: D.02. Auditory System

Support: DIRP, NIMH/NIH

Title: Activity of neurons in lateral prefrontal cortex during performance of an auditory short-term memory task

Authors: *B. H. SCOTT[1], P. YIN[2], L. H. LEE[1], A. BROWN[1], M. MISHKIN[1];
[1]Lab. Neuropsychol, NIMH, Bethesda, MD; [2]Neural Systems Laboratory, Inst. for Systems Res., Univ. of Maryland, College Park, MD

Abstract: Short-term memory (STM) for visual stimuli has been shown to engage both prefrontal cortex (PFC) and the modality-specific cortical areas that support visual perception. Although monkeys can perform auditory STM tasks, their ability is limited relative to that in vision and appears to depend on passive retention of a stimulus trace. Neurons in rostral superior temporal cortex (rSTC) carry a potential correlate of this sensory trace, but the contribution of the dorsolateral PFC (dlPFC) to this process is unresolved. We recorded single-unit activity in dlPFC (the caudal portion of the principal sulcus, predominantly its dorsal bank) while a monkey performed a serial delayed-match-to-sample (DMS) task. On each trial, the monkey grasped a bar to initiate the presentation of a sample sound (~300 ms duration), followed by 0-2 nonmatch sounds (delay interval ~1 s), before the sample was presented again as a match; the monkey released the bar to indicate a match. Of 101 recorded units, 39 responded to sound, some at a surprisingly short onset latency (median 44 ms, minimum 22 ms). Firing rate was stimulus-selective in 30% of responsive units. Two classes of task-related effects were identified: First, the auditory response was modulated by task context in one-third of units, with 10/39 showing match suppression (relative to the sample presentation), and 3/39 showing match enhancement. However, suppression and enhancement were equally prevalent for match or nonmatch sounds, indicating that these effects were not stimulus-specific. Second, activity during the first delay period was shifted from baseline in one-half of units, with delay suppression (14/39) being more common than delay enhancement (4/39). Delay suppression was typically sustained throughout the trial duration, and could possibly drive the same effect previously observed in rSTC. Other forms of delay activity were qualitatively different and more variable in dlPFC than in rSTC. As in rSTC, there was little evidence for a sustained, stimulus-specific sensory trace in the delay activity of dlPFC neurons. Unlike rSTC, suppression and enhancement of match responses in dlPFC were not stimulus-specific, but seemed to reflect a generalized modulation of responses

following the sample. Responses in some units were strongly dependent on task engagement. In addition, firing rate in dlPFC was often modulated around the time of the motor response, which was rarely seen in rSTC. These observations confirm that dlPFC receives short-latency auditory inputs, but suggest that activity in this region also encodes domain-general aspects of the serial DMS task.

Disclosures: **B.H. Scott:** None. **P. Yin:** None. **L.H. Lee:** None. **A. Brown:** None. **M. Mishkin:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 57.11/J5

Topic: D.02. Auditory System

Support: 093292/Z/10/Z

Title: How the brain discovers patterns in sound sequences

Authors: ***M. CHAIT**[1], N. BARASCUD[1], T. PETSAS[1], M. PEARCE[2], K. FRISTON[1]; [1]UCL, London, United Kingdom; [2]QMUL, London, United Kingdom

Abstract: Patterns or regularities in on-going sound sequences are key cues to understanding complex auditory environments. The pattern of sound often conveys the identity and state of objects within the scene and also enables the listener to predict future events, supporting efficient interaction with the surrounding environment. This presentation will review our recent behavioral and brain imaging findings that demonstrate just how sensitive we are to complex sound patterns, including those that we have never previously encountered and, indeed, maybe unlikely to encounter outside of the laboratory. Our findings suggest that the auditory brain is a remarkably well-tuned 'pattern seeker', continuously scanning the unfolding auditory input for regularities, even when listeners' attention is focused elsewhere. Brain responses reveal online processes of evidence accumulation - dynamic changes in tonic activity precisely correlate with the expected precision or predictability of ongoing auditory input -both in terms of deterministic (first-order) structure and the entropy of random sequences. Source analysis demonstrates an interaction between primary auditory cortex, the hippocampus and inferior frontal gyrus in the process of 'discovering' the regularity within the ongoing sound sequence. The results are consistent with precision based predictive coding accounts of perceptual inference and provide

compelling neurophysiological evidence of the brain's capacity to encode high order temporal structure in sensory signals.

Disclosures: **M. Chait:** None. **N. Barascud:** None. **T. Petsas:** None. **M. Pearce:** None. **K. Friston:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

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Topic: D.02. Auditory System

Support: DFG SFB/TR 62

BmBF/NSF

Title: Cortico-striatal interactions during reversal learning with different reinforcement schedules in a two-way active avoidance task

Authors: ***A. L. SCHULZ**[1], M. L. WOLDEIT[1], A. I. GONCALVES[2], M. BROSCH[2], F. W. OHL[1,2];
[1]Leibniz Inst. for Neurobio., Magdeburg, Germany; [2]Otto von Guericke Univ., Magdeburg, Germany

Abstract: Due to their demonstrated importance in reinforcement learning and reward processing, cortico-striatal interactions should also take part in serial reversal learning that demands multiple switches of stimulus-action contingencies and henceforth represents a scenario for cognitive flexibility. While computational models, like the actor-critic variant of temporal difference learning, implicitly assume a modification of cortico-striatal functional coupling in the reinforcement schemes, it is not clear if a task that encompasses multiple contingency changes would repeatedly adjust this cortico-striatal coupling or is based on a different mechanism. Adding to this, reinforcement learning models continuously have fallen short of explaining the different learning dynamics found in many serial reversal tasks for animals. A neurophysiological understanding of sensory cortico-striatal interactions during learning is still in its early stages and previous work has not yet addressed the question how these interactions are modulated in serial reversal tasks. Therefore we trained Mongolian gerbils in an auditory discrimination two-way-active-avoidance task with multiple contingency reversals, employing frequency modulated tones as conditioned stimuli. We used two reinforcement schedules: one

group received symmetric aversive reinforcement on false alarm and miss responses, while the second group only received punishment for misses. Simultaneously we recorded local field potentials from the auditory cortex and ventral striatum and assessed functional coupling of both areas during acquisition and reversal phases by analyzing the neuronal coherence. We expect that cortico-striatal interactions during serial reversal learning of auditory discrimination problems will have a different time course than during the initial discrimination phase. Since preliminary data show that both reinforcement schedules produce different superordinate strategies in the serial reversal task, it is expected that this will also influence auditory cortico-striatal functional coupling.

Disclosures: **A.L. Schulz:** None. **M.L. Woldeit:** None. **A.I. Goncalves:** None. **M. Brosch:** None. **F.W. Ohl:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 57.13/J7

Topic: D.02. Auditory System

Support: Internal support from the University of Iowa

Title: Auditory discriminative fear conditioning utilizing a visual secondary reinforcer

Authors: *J. M. BOWDEN, S. J. CONNELL, A. POREMBA;
Psychology, Univ. of Iowa, Iowa City, IA

Abstract: Learning to escape aversive stimuli and effectively predicting the consequences of different cues and responses provides animals with an increased chance of survival. Discriminative avoidance conditioning affords the opportunity to examine these specific behaviors. The present experiment investigated the influence of a secondary reinforcer on an auditory discriminative active avoidance conditioning task. Building on the work of Poremba and Gabriel (1997), originally conducted in rabbits, an adaptation of the discriminative active avoidance paradigm was implemented using male Sprague-Dawley rats (N=35). Pilot studies were used to optimize task contingencies and parameters for learning in rats, e.g., separate versus combined footshock and tailshock, delay interval duration, and auditory stimulus characteristics. In the final paradigm, animals were trained to avoid a signaled mild tail and foot shock (US) by spinning a small wheel at least 22.5 degrees using their forepaws as the conditioned response (CR) during the high-frequency auditory cue, the positive conditioned stimulus (CS+). A second

low-frequency auditory cue signaled safety, the negative conditioned stimulus (CS-), and the absence of shock. CS+ and CS- trials were presented with equal probability in random order with an intertrial interval of 20 seconds. The CS+ always preceded the US, and CS presentations had a duration of 3 seconds and were terminated when a response was made. Eleven of the male rats were trained using a 1 s light as a secondary reinforcer following correct responses to the CS+ (successful avoidance of the shock). Preliminary data show increased performance in the group of subjects trained with the secondary reinforcer on three measures. Animals trained without a secondary reinforcer learned the task within 30-80 days and animals trained with the light cue acquired the task within 8-25 days. A higher proportion of animals learned using the secondary reinforcer, with 72.7% successfully acquiring the task compared with 12.5% without the light. These animals also showed significantly steeper learning curves, and higher asymptotic discrimination performance. The results suggest that a visual secondary reinforcer enhances learning during an auditory discriminative avoidance conditioning task. This task will be used to expand exploration of the active avoidance neural circuitry and investigate its implications regarding the cooperation and organization of processing across multiple sensory systems.

Disclosures: J.M. Bowden: None. **S.J. Connell:** None. **A. Poremba:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

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Program#/Poster#: 57.14/J8

Topic: D.02. Auditory System

Support: NIH-R01DC010145

R01DC013979

NSF grant BCS 1262297

Title: Neural correlates of audiomotor map learning

Authors: *M. THOMPSON[1,2], A. B. HERMAN[3], D. C. HARRELL[3], J. HOUDE[3], S. NAGARAJAN[3];
[1]UCSF, San Francisco, CA; [2]UC Berkeley-UCSF Joint Grad. Program in Bioengineering, Univ. of California, Berkeley, Berkeley, CA; [3]Univ. of California, San Francisco, San Francisco, CA

Abstract: Typically, sensorimotor learning studies have used visuomotor adaptation or speech adaptation. In this study, we sought to derive more general principles about audiomotor control

by examining auditory feedback processing through the use of neuroimaging and a magnetoencephalography imaging (MEGI)-compatible touchpad. Initial study was conducted with non-speech tone stimuli, and we are currently extending this paradigm to speech sounds. Subjects learned a novel audiomotor map task, eventually developing an internal model of auditory feedback triggered in response to locations they touched on the touchpad. Following training, 15 subjects were given a target cue via headphones and attempted to reproduce it by touching the corresponding area on the touchpad and subsequently receiving feedback. 10 out of 15 subjects demonstrated better-than-chance responses, improving upon their initial performance, indicating stable learning. In addition to demonstrating an increased rate of correct responses, evidence for development the internal model of auditory expectations was seen in significant MEGI differences in post-learning neural response between correct and incorrect responses. These differences showed efference copy-based suppression in the auditory cortex in correct feedback and error/conflict monitoring in frontal areas in incorrect feedback. Notable frontal lobe suppression was also found in high gamma band in correct responses compared to when the subject passively listened to tones (n=4). Additionally, some left temporal lobe suppression was seen in typical correct responses with expected auditory feedback compared to "surprise" trials in which, after training, subjects correctly identified the location for the probe tone but received unexpected auditory feedback (n=4). Increased activation in areas responsible for error monitoring in incorrect trials and suppression in sensorimotor areas during correct responses is consistent with efference copy comparison. We are now examining speech stimulus learning and adaptation behavior with this paradigm. By investigating speech and non-speech auditory feedback processing without speech motor feedback, we can elucidate more general auditory-motor control effects.

Disclosures: **M. Thompson:** None. **A.B. Herman:** None. **D.C. Harrell:** None. **J. Houde:** None. **S. Nagarajan:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 57.15/J9

Topic: D.02. Auditory System

Support: UC Irvine School of Medicine Bridge Fund

NIH NINDS NS060993 K23

NINDS R37NS21135 and the Nielsen Corporation

The National Defense Science and Engineering Graduate Fellowship

Title: Human auditory cortical response to low-level acoustic features shifts during perceptual enhancement

Authors: *C. HOLDGRAF[1], W. DE HEER[2], B. PASLEY[3], J. RIEGER[3], J. LIN[4], N. CRONE[5], R. T. KNIGHT[3], F. THEUNISSEN[3];
[1]Helen Wills Neurosci. Inst., Berkeley, CA; [2]Dept. of Psychology, [3]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA; [4]Neurol. Sch. of Med., Univ. of California, Irvine, Irvine, CA; [5]Dept. of Neurol., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Listening to human speech is a hierarchical process in which low-level features such as spectrotemporal energy are combined to create high-level features such as words. This is often treated as a "bottom-up" series of feature extraction steps. However, top-down signals from cortical regions that respond to "high-level" features also influence regions that process lower-level stimulus features. The information we receive from the world is often noisy or incomplete, and these high-level inputs may facilitate making sense of this noisy information by "filtering out" unwanted streams of information or "filling in" missing information in the stimulus. However, there is little evidence to show that high-level auditory/linguistic features affect the representation of specific low-level features in auditory cortex. The present study provides evidence for a shift in the auditory cortical response to low-level acoustic features during a hierarchical perceptual enhancement task. We filtered spoken sentences from the TIMIT database using the modulation transfer function, rendering the sentences unintelligible. We presented these sounds both before and after presentation of an unfiltered complete sentence to patients implanted with electrocorticography (ECoG) grids. We focused on the high gamma band (HG; 80-175 Hz) of the ECoG signal in 7 patients with subdural grids over perisylvian cortices. After hearing the unfiltered sound, comprehension of the following filtered version is enhanced due to the experience with high-level stimulus features (here referred to as "hierarchical perceptual enhancement"). We investigated the influence of high-level sentence context on the neural response to the same sentence's degraded version by constructing linear encoding models of electrode response to acoustic features and comparing model fits to pre- and post-unfiltered stimuli. We found that listening to a filtered sound after hearing its unfiltered version alters auditory cortex response to low-level acoustic features, rendering them more similar to the response to unfiltered speech. We also show that acoustic models built from unfiltered stimuli provide better predictions about brain activity in the post-unfiltered trial. Finally, receptive fields fit on the neural response to filtered speech during perceptual enhancement respond more strongly to speech stimuli than their unintelligible speech counterparts. The results may reflect an automatic process of hierarchical perceptual enhancement that shapes the neural response to low-level acoustic features in order to facilitate processing of speech in noise.

Disclosures: **C. Holdgraf:** None. **W. De Heer:** None. **B. Pasley:** None. **J. Rieger:** None. **J. Lin:** None. **N. Crone:** None. **R.T. Knight:** None. **F. Theunissen:** None.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 57.16/J10

Topic: D.02. Auditory System

Title: Cortical neuroplasticity in single-sided deafness before and after cochlear implantation

Authors: *H. GLICK, A. SHARMA;
Speech, Language, & Hearing Sci., Univ. of Colorado, Boulder, CO

Abstract: The extent to which sensory pathways reorganize in single-sided deafness (SSD) is not well understood. While cochlear implantation has proved beneficial in bilateral pediatric deafness, there is currently little evidence demonstrating the efficacy of cochlear implantation in children with SSD. The purpose of this study was to examine changes in cortical development and neuroplasticity in adults and children with SSD before and after cochlear implantation. High-density 128-channel electroencephalography (EEG) was used collect cortical auditory, visual, and somatosensory evoked potentials (CAEP, VEP, SSEP) in adults and children with SSD before and after implantation. Behavioral correlates of speech perception in noise were also measured. Prior to implantation, high-density EEG showed abnormal auditory activation patterns and evidence of increased cognitive load and cross-modal re-organization. Post-implantation, there is clear evidence of morphological changes in the auditory cortical responses consistent with increases in auditory speech perception in noise with some residual evidence compensatory changes in neural resource allocation. With further research, it is possible that markers of cross-modal re-organization or evidence of cognitive load may help predict cochlear implant outcomes in SSD patients and guide the intervention and rehabilitation process.

Disclosures: H. Glick: None. **A. Sharma:** A. Employment/Salary (full or part-time):; University of Colorado.

Poster

057. Auditory Processing: Adaptation, Learning, and Memory

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Program#/Poster#: 57.17/J11

Topic: D.02. Auditory System

Support: Natural Science Foundation of China Grant 31230030

Title: Perceptual learning of auditory frequency discrimination transfers to untrained frequencies with TPE training

Authors: *C. YU[1], Y.-Z. XIONG[1], Y.-X. ZHANG[2];
[1]Peking Univ., Beijing, China; [2]Beijing Normal Univ., Beijing, China

Abstract: Perceptual learning is typically specific to the trained stimuli features. However, recent research on visual learning has shown that such stimulus specificity could be removed with a training-plus-exposure (TPE) procedure, in which subjects are exposed to the to-be-transferred stimulus feature via practicing an irrelevant task (Zhang et al., 2010). Here we extended this visual training method to auditory perceptual learning to study the transfer of learning of auditory tone frequency discrimination (FD). FD learning is known to be specific to the trained tone frequency. In addition, FD is unaffected by learning of temporal interval discrimination (TID) with the same stimuli (Wright, et al, 2010). We thus used the TID task to provide exposure to untrained tone frequencies. In the first experiment, listeners were trained with FD at 1 kHz plus exposure at 4 kHz via TID, which improved FD significantly at both frequencies. As a baseline, listeners trained with 1-kHz FD only did not show learning transfer to 4 kHz, and listeners trained with 4-kHz TID did not improve on FD at either frequency. The results indicate that TPE enabled complete transfer of FD learning from 1 kHz to 4 kHz. We next asked whether the transfer-enabling effect of TPE training is contingent on the neural coding mechanisms. Auditory nerve responses can phase-lock to the sound waveform up to about 5 kHz, rendering higher frequencies to be encoded by a 'place code', while lower frequencies are encoded temporally. In the second experiment, we used TPE to enable transfer of FD learning from 6 kHz to 1 kHz, across the gap between the temporal and place coding mechanisms. The TPE group improved significantly at both 1 and 6 kHz, while the baseline 6-kHz FD training group did not improve at 1 kHz, and the 1-kHz TID training group did not improve on FD at either frequency. The transfer of TPE group to 1-kHz was similar to learning obtained by directly training 1-kHz FD. Thus, TPE enabled complete learning transfer across different coding mechanisms. The current results, combined with previous observations of visual learning transfer, suggest that learning under TPE possibly engages high-level processes that function regardless of modality and the specific neural encoding mechanisms involved.

Disclosures: **C. Yu:** None. **Y. Xiong:** None. **Y. Zhang:** None.

Poster

058. Retina: Photoreceptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: D.04. Vision

Support: NIH Grant EY020542

Title: Trafficking of HCN1 in the early secretory pathway: Discovery of two counteracting ER trafficking signal using Xenopus photoreceptors as a model system

Authors: *S. A. BAKER, J. G. LAIRD, D. M. YAMAGUCHI, Y. PAN;
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Abstract: The hyperpolarization-activated cyclic nucleotide gated family of channels (HCN1-4) play diverse roles throughout the nervous and cardiac systems; they can function as pacemakers, shape resting membrane potential, modulate synaptic output, or integrate dendritic signaling. Rod photoreceptors selectively express HCN1, and it functions to carry a feedback current that shapes visual responses under medium and bright light conditions. Rod photoreceptors are linearly compartmentalized with a highly specialized ciliary compartment dedicated to light detection. HCN1 is excluded from this compartment and instead is found concentrated in the soma, known as the inner segment. As part of a long-standing interest in dissecting protein trafficking pathways in photoreceptors we have investigated the mechanisms controlling the localization of HCN1. We first investigated the role of the HCN1 accessory subunit, TRIP8b. In the retina of TRIP8b KO mice, HCN1 levels are reduced but trafficking to the inner segment plasma membrane, and visual function, is maintained. Therefore, we conclude that TRIP8b is not essential for HCN1 trafficking in photoreceptors. To identify other contributors to HCN1 trafficking we examined the localization of a series of HCN1 mutants expressed in the rods of transgenic tadpoles (Xenopus laevis). We identified a di-arginine motif in the C-terminus of HCN1 just distal to the cyclic nucleotide binding domain that functions to retain HCN1 in the ER. As expected, mutating this motif resulted in increased surface expression of HCN1. A second trafficking signal in the N-terminus of HCN1, near the first transmembrane domain, was found to be necessary for ER export. This work leads to a model where these two signals work together to maintain a proper equilibrium of HCN1 levels between ER and the plasma membrane. Since exit from the ER is such an early step in any trafficking pathway, these signals are likely to contribute to the balance of HCN1 trafficking in other cell types.

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Poster

058. Retina: Photoreceptors

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Topic: D.04. Vision

Support: NINDS Intramural Program

Title: Development of cone opsin expression in a transgenic zebrafish line with crx-driven trβ2

Authors: *S. S. PATTERSON[1,2], T. YOSHIMATSU[2], T. SURESH[1], R. F. NELSON[1];
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Abstract: In the transgenic zebrafish line crx:MYFP-2A-trβ2, the highly conserved cone-rod homeobox (crx) gene drives thyroid hormone β2 (trβ2) expression (Suzuki et al., 2013). Crx is expressed early in retinal progenitors that later differentiate into photoreceptors and trβ2 is a necessary for subsequent red cone development. The result is primarily L-opsin expression with scarce G-, B- and UV-opsin expression. This transgenic provides the opportunity to study photoreceptor development and crx, a gene implicated in human photoreceptor degeneration. Larvae were spawned from an outcross of crx:trβ2 and wildtype (WT) adults and phenotyped by MYFP pupil fluorescence. Larval eyes aged 5 - 12 days post-fertilization (dpf) were isolated and perfused with oxygenated MEM containing 20mM L-Aspartate or 10mM CNQX to isolate the photoreceptor (PIII) and ON-bipolar (b2) responses, respectively. Microelectrode ERG responses were recorded to 300ms light stimuli presented at 9 wavelengths (330-650nm), each at 7 irradiances. Spectral sensitivities were calculated with an ERG model summing 4 Hill functions, one for each zebrafish cone type. To examine Vitamin A2 expression, a 5th Hill function was added using a 600nm λmax. Despite early L-opsin dominance, a small 330nm PIII response occurred in crx:trβ2 at 5dpf. The 330nm b2 response was absent\. By 12dpf, the PIII and b2 crx:trβ2 responses increased. All crx:trβ2 amplitudes were smaller than WT amplitudes. Crx:trβ2 PIII spectra showed increasing short wavelength sensitivities and decreasing long wavelength sensitivity from 5 to 12 dpf. At 12dpf, MYFP expression was still widespread but immunostaining showed UV and blue opsin expression in the peripheral retina.600nm A2 sensitivity occurred more often in crx:trβ2 spectra than in WTs. In both genotypes, the percent of spectra with 600nm A2 sensitivity showed a similar downward trend from 5 to 12dpf crx:trβ2 decreasing from 79% to 44% and WT from 62% to 14%. Zebrafish photoreceptor differentiation is normally complete by 4dpf. However, crx:trβ2 spectra and opsin expression suggest blue and UV cones are continuing to develop between 5 and 12dpf. Vitamin A2 is present in wildtype larvae, elevated in crx:trβ2 and decreases through development in both genotypes.

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Poster

058. Retina: Photoreceptors

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Title: Statistical analysis of zebrafish locomotor response

Authors: *Y. LIU[1], R. CARMER[2,3], G. ZHANG[2], P. VENKATRAMAN[2], S. A. BROWN[2], C. P. PANG[5], M. ZHANG[6,7], Y. F. LEUNG[4], P. MA[1];
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Abstract: Zebrafish larvae display rich locomotor behaviour upon external stimulation. This locomotor behaviour can be simultaneously tracked from many larvae systematically arranged in multi-well plates. The resulting time-series locomotor data have been used to reveal new insights into neurobiology and pharmacology. However, the data are high-dimensional, and are affected by multiple intrinsic and extrinsic factors. These issues pose a statistical challenge for comparing larvae exposed to different stimuli, which cannot be efficiently handled by simple parametric analyses. To address this gap, this study has evaluated the Hotelling's T-squared test for analyzing a visually-driven locomotor behaviour named the visual motor response (VMR). This test is the generalization of the conventional t-test, and is congruent with comparing locomotor profiles from a defined period of time. With this test, various comparisons were conducted using VMR data collected from three wild-type (WT) strains: AB, TL and TLAB, from 3 to 9 days postfertilization. The comparisons show that different WT strains responded differently to light change at different developmental stage, and that sequential technical repeats were similar, if they were collected with the same parameters. Furthermore, the performance of this test was evaluated by a power analysis, which indicates that the test is sensitive for detecting differences between experimental groups with sample number that is amenable to the existing multi-well

plate platform. In addition to the evaluation of this test, our study also investigated the effects of various intrinsic and extrinsic factors that might affect the VMR by multivariate analysis of variance. The results indicate that the larval activity was generally affected by four major factors: stage of the larvae, light stimulus, their interaction, and location in the plate. Nonetheless, different factors affected larval activity to a different extent over time, as indicated by a dynamical analysis of the activity at each second by a conventional analysis of variance. Intriguingly, this analysis showed a negligible effect on larval activity by biological and technical repeats, and their effect size was fairly constant regardless of the light illumination. Thus, the results hint at the possibility to combine the data obtained from biological and technical repeats to enhance the statistical power of analysis, despite the perceived variations between replicates. Together, these investigations have established a statistical framework for analyzing VMR data, a framework that should be generally applicable to other locomotor data with similar structure.

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Poster

058. Retina: Photoreceptors

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Title: Understanding the contributions of photoreceptors to the visual motor response

Authors: ***P. VENKATRAMAN**[1], R. CARMER[2], C. PANG[3], M. ZHANG[4], Y. LEUNG[5]; [1]Biol. Sci., [2]Statistics, Purdue Univ., West Lafayette, IN; [3]Ophthalmology and Visual Sci., Chinese Univ. of Hong Kong, Hong Kong, Hong Kong; [4]Joint Shantou Intl. Eye Center, Shantou Univ. & The Chinese Univ. of Hong Kong, Shantou, China; [5]Biol. Sciences; Biochem. and Mol. Biology, IU school of Med., Purdue Univ. and Indiana Univ. Sch. of Med., West Lafayette, IN

Abstract: Visual Motor Response (VMR) is a startle locomotor response elicited by the eye; and can be observed in larval zebrafish, when they are presented with varying intensities of light stimuli. Two components of the VMR: ON and OFF responses, arise due to light or dark stimulus. The Light ON response consists of only a rapid response phase which is evident in a scale of seconds; whereas the Light OFF response, in addition to the rapid phase, consists of a sustained response phase which is evident in a scale of minutes. While VMR has been increasingly used to study chemical screening of compounds, the underlying cellular circuitry has not been fully understood. The research reported here has the goal to identify specific contributions of photoreceptors to the VMR. To study how VMR was affected by lack of cones, the *no optokinetic response f*^{w21} (*nof*) zebrafish mutant lacking functional cones was used. Wildtype (WT) and *nof* larvae were presented with light stimuli of varying intensities and the locomotor movements were tracked using an infrared camera. The average locomotor activity was plotted separately for Light ON (transition from darkness to light) and Light OFF responses (transition from light to darkness). Additionally *chokh* mutants were included in the study. Since they are eyeless, any response coming from *chokh,* would be of extra-ocular origin. Light stimuli with intensities ranging from log 0 (1440 lux) to log -3.21 (0.896 lux) were presented to the larvae and the VMR was recorded on 6 dpf (days post fertilization), 7 dpf and 8 dpf. At log 0 and log -2 stimulus intensities, the WT larvae showed a characteristic rapid response phase at stimulus onset. This was absent in the *nof* mutants, suggesting that cones could mediate the Light OFF rapid response phase at high intensity of light. Interestingly at lower intensities, such as log -2.81, the *nof* Light ON responses were comparable to the WT. This suggests that at lower light intensities, rapid response phase of Light ON could be mediated by rods. The *chokh* mutants did not show rapid responses to all the light intensities tested. However, they show a delayed response to stimuli suggesting that these responses could be from extra-ocular sources.

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Poster

058. Retina: Photoreceptors

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DFG Ha5323/5-1

Title: Psychophysical estimation of cone connectivity and noise in the human retina

Authors: ***A. S. MCKEOWN**[1], K. S. BRUCE[2], W. M. HARMENING[3], L. C. SINCICH[2]; [1]Vision Sci., Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama Birmingham, Birmingham, AL; [3]Univ. of Bonn, Bonn, Germany

Abstract: Detection of photopic stimuli occurs when light captured by a cone photoreceptor produces a signal that downstream neurons can separate from ongoing cellular noise. Stimulating multiple cones simultaneously should alter the relationship between stimulus intensity and the probability of seeing, changes that would be reflected in the psychometric function of the detection process. One parameter that defines this function, threshold, may shift in one of two ways depending on the type of summation exhibited between detectors. When signals from two adjacent cones feed into a single neuron, perceptual threshold may be reduced by a factor of 2 if the inputs add linearly. Alternatively, if cone signals are sampled by two independent neurons, threshold may instead be reduced by a factor of √2 (2-detector summation). Another parameter, slope, may change systematically as noise in the combined inputs is reduced. To learn if these features of cone signaling can be measured psychophysically, we quantified increment sensitivity of single and multiple cones with microstimulation in 3 subjects. We used a multiwavelength adaptive optics scanning laser ophthalmoscope to image and stimulate cones in a 1.2° retinal field. A 130 μs, 543 nm, cone-sized (~3.6 μm) stimulus flash of varying intensity was delivered to each cone in a pair or triplet simultaneously, or to each cone individually, in pseudo-randomly interleaved trials. Psychometric functions were generated by fitting a logistic function to the frequency of seeing data by parametric bootstrapping. In 10 cone pairs tested, we found that single cones had variable perceptual thresholds, and that the slope of the psychometric function increased reliably when multiple cones were stimulated together, indicating a reduction in perceptual noise. We also observed a shift towards lower thresholds, with respect to the individual cones in the pair. After weighting by cone threshold, we computed predicted 2-cone functions from the single cone data that followed either a linear or a 2-detector model, and compared these predictions to observed 2-cone data. Six of the cone pairs exhibited significantly linear summation, three unambiguously followed a two-detector model, and one pair did not match either. In 4 cone triplets tested, summation fell between linear and two-detector predictions, suggesting a mixture of connectivity among these cones. Their psychometric functions had steeper slopes than the pairs, indicating a further reduction of noise with an additional receptor. Our results suggest that cone connectivity and physiological noise can be probed at the single cell level in the human retina through psychophysical measurements.

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Poster

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DFG Ha5323/5-1

Eyesight Foundation of Alabama

Title: Cone signal summation varies with inter-cone distance in the human retina

Authors: *K. S. BRUCE[1], W. M. HARMENING[2], W. S. TUTEN[3], A. S. MCKEOWN[4], A. ROORDA[3], L. C. SINCICH[4];
[1]Vision Sci., Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Bonn, Bonn, Germany; [3]Univ. of California Berkeley, Berkeley, CA; [4]Univ. of Alabama Birmingham, Birmingham, AL

Abstract: The nature of cone connectivity in the retina would suggest that any two cones may be connected to either one or two downstream neurons of a single class. If two cones send signals to one neuron, they may act as one detector, and threshold may be reached by linear summation. If so, only ½ as much light needs to be delivered to each of the two cones as is needed for one cone to reach threshold. Alternatively, if two cones transmit signals to separate neurons, a 2-detector model suggests that 2-cone thresholds would be reached with $1/\sqrt{2}$ the amount of light required for single cones. We hypothesized that inter-cone distance and the type of summation would be related, with more separated cones being more likely feed onto different neurons, thus exhibiting $1/\sqrt{2}$ summation. To examine this, cones were imaged and stimulated using a multiwavelength adaptive optics scanning laser ophthalmoscope that tracked eye motion over a 1.2° patch of retina. We selected cone pairs for targeted delivery of a 45 arcsec spot of 543 nm light, flashed for 130 μs. To measure the effect of inter-cone distance, one anchor cone was common to every pair, while a second cone was selected by systematically varying the inter-cone distance. Increment thresholds were measured 5 times using a self-paced, 20 trial staircase method, and

were interleaved for 3 conditions: stimulation of the anchor cone, the distant cone, or both cones simultaneously. Subjects (n=5) had normal color vision. Cone pairs were located 1.4-3.6° from the fovea. As we found previously, single cone thresholds sometimes differed significantly from one another. To account for this, weighted single cone thresholds were computed to estimate summation. Of 45 pairs tested, 9 demonstrated linear summation (20%; $p<0.05$), 22 showed 2-detector summation (49%; $p<0.05$), and 14 had summation that was non-significant to either model (31%). At each of 6 tested retinal locations, mean cone spacing values were obtained by measuring the inter-cone distance of 500 cones. The inter-cone distance for each pair was normalized by the mean cone spacing at the site, allowing us to analyze our data independent of cone-spacing changes due to eccentricity. When cone signal summation was examined in relation to inter-cone distance, we found summation became exclusively 2-detector as inter-cone distance increased. All 9 linearly summing cone pairs were located within 1.5 cone distances of one another, while the 22 two-detector pairs spanned a range of 0.7-4.4 cone distances. These results suggest that at greater inter-cone distances, cones are likely signaling to more than one downstream retinal neuron, and that this feature of connectivity can be probed psychophysically.

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Poster

058. Retina: Photoreceptors

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Topic: D.04. Vision

Title: Rgma inhibits angiogenesis via neogenin

Authors: *K. HARADA, Y. FUJITA, T. YAMASHITA;
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Abstract: Vascular endothelial growth factor (VEGF) signaling in pathological angiogenesis has been studied; however, in some patients with Age-related Macular Degeneration (AMD) become refractory to further anti-VEGF therapy. The repulsive guidance molecule A (RGMa) was originally identified as an axon repellent in the visual system. It has diverse functions in both developing and adult central nervous system. These functions are mediated by binding of RGMa to its receptor, neogenin. Previously, Lejmi et al. (2008) reported that Netrin-4 inhibited

angiogenesis binding to neogenin. Therefore, we focused on RGMa, a Neogenin receptor, similar manner in Netrin-4 verifying therapeutic effect in AMD. Here, we show that RGMa is involved in angiogenesis and can suppress new blood vessel formation both *in vitro* and *in vivo*. Treatment of human umbilical artery endothelial cells (HUAEC) with recombinant RGMa inhibited vascular endothelial growth factor (VEGF)-induced tubular formation and migration. Knockdown of neogenin in HUAEC abolished the inhibitory effect of RGMa on tubular formation. Thus, we concluded that RGMa is an inhibitory molecule for angiogenesis and suggest that its manipulation could be an alternative for therapeutic strategies in AMD.

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Poster

058. Retina: Photoreceptors

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Topic: D.04. Vision

Support: CONACYT 178526

PAPIIT IN210913

Title: Immunoreactivity of the protein Cryptochrome (CRY) in retina of crayfish Procambarus clarkii

Authors: *R. LOREDORANJEL, E. ESCAMILLA-CHIMAL;
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Abstract: Previous works from this laboratory, by immunohistochemistry and WB have shown the presence of the protein cryptochrome (CRY) in both the optic lobe and brain of the crayfish P. clarkii. The presence and participation of CRY in extraretinal synchronization of the circadian system of P. clarkii subsequently has been confirmed a posteriori by other research groups (1). However, despite that both vertebrates and invertebrates CRY has also localized retinal structures and since we have proposed to the retina as a possible pacemaker system multioscillatory crayfish (2). The aim of this work is the location of the CRY protein in retinal structures. To accomplish the above it was made the immunolocalization of CRY in the retina of P. clarkii. Adult animals fed ad libitum, maintained at constant temperature and oxygenation were synchronized in photoperiod light dark (LD) 12:12. The dissection was performed in ZT0 (on light), the retinas were fixed with 4% paraformaldehyde, cryopreserved with 30% sucrose,

included in Tissue Tek's OCT and using a cryostat retinal sections were made to 10µm. Immunofluorescence was performed with a primary antibody anti-*Drosophila* CRY (1:50) and a secondary antibody Dy light 594 (1:100) and the nuclei were counterstained with DAPI (1:1000). A pre-absorption with the blocking CRY-peptide was performed as a control. The results showed that there immunoreactivity in the retina of crayfish Procambarus clarkii specifically was in the rhabdoms, all controls were negatives. As the retina a putative pacemaker of the biological clock, we can propose that CRY can be linked to animal photoreception and to be part of the clock proteins, as in other organisms. 1)Sullivan et al., (2009). Chron. Biol. Int. 26(6):1136-1168. 2)Velázquez-Amado R. M., et al., (2013). LVI Congress of Sociedad Mexicana de Ciencias Fisiológicas.

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Poster

058. Retina: Photoreceptors

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Conselho Nacional de Desenvolvimento Científico e Tecnológico

Title: Toxicity of intravitreal administration of anti-VEGF drugs to the retina

Authors: *S. ALLODI[1], A. O. FONTES[2], R. M. JAPIASSU[3], G. HOLLMANN[2], M. A. FUSCO[4];

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Abstract: VEGF - Vascular Endothelial Growth Factor - is closely linked to the genesis of proliferative vessel disorders of the retina, such as age-related macular degeneration, diabetic retinopathy and retinopathy of prematurity. Neutralizing VEGF drugs have become the main therapeutic choice for the treatment of these conditions. Lucentis® (ranibizumab) is a smaller fragment of a humanized antibody against VEGF and Eylia® (aflibercept) is composed by fragments of VEGF receptors. Studies have revealed that the target of VEGF may include not only endothelial, but also neural cells, and tachyphylaxis, a decreased therapeutic response after repeated administrations of a medication, has been described for these drugs. Therefore, it is necessary to evaluate the toxicity of the intravitreal administration of neutralizing VEGF drugs to the retina on the cellular and molecular basis. A single dose of Lucentis, Eylia or sham (saline) was injected intravitreally in five rabbits per group. Contralateral eyes were left untreated (control). After fifteen days, the retinas were dissected for immunoblotting assays using anti-VEGF, anti-FLK-1 (VEGF receptor), anti-GFAP and anti-phosphohistone H3 (PH3). ANOVA followed by Tuckey test showed no statistically difference between the groups in VEGF ($p=0.65$) and FLK-1 ($p=0.56$), showing similar tendency between them. GFAP showed an increased expression in the Lucentis group in relation to control ($p<0.05$) and Eylia group ($p<0.05$), while PH3 showed an increase in Lucentis ($p<0.05$) in relation to sham. The fact that similar quantity of VEGF was observed in retinas treated with anti-VEGF drugs led to the hypothesis of an overexpression in the treated retinas, since anti-VEGF drugs should knock out the constitutive VEGF present in normal retinas. Thus, the similar amounts of VEGF and FLK-1 both in control and treated retinas may be due to an attempt of the treated retinas to return to the constitutive levels of VEGF needed to maintain the protection of neural cells, since the increase in GFAP and PH3 content in Lucentis may be indicating gliosis, a neurodegenerative condition in the retina. Because the role of constitutive VEGF in the retina is still unkown, the use of neutralizing VEGF drugs should be considered with care.

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Poster

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Title: Role of sigma receptors in the viability of retinal pigment epithelial cells -- studies using the crispr-cas9 system

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Abstract: Sigma receptors play an important role in cellular viability. It has been shown that activation of the Sigma-1 receptor (S1R) has a protective effect in the survival of some neurons. While much less is known about the function of the Sigma-2 receptor (S2R) which is yet to be cloned, it has been reported that activation of S2R induces apoptosis in cancer cells. In the retina, S1R is abundant in ganglion cells and has been shown to be protective. S1R is also present in other retinal neurons although its function remains unclear. While transcripts of S1R were found in the retinal pigment epithelium (RPE) of mouse retina, the presence of the protein product of sigma receptors, in particular, S2R, has never been clearly demonstrated. The function of S1R and S2R in the RPE remain underexplored. Using a unique probe for photoaffinity labeling, we detected high amounts of both sigma-1 and sigma-2 receptors in crude preparations of bovine retinas as well as retinal pigment epithelium (RPE). Binding assay using [3H]-DTG in the presence of non-radioactive (+)-Pentazocine also confirmed high levels of S2R in RPE. Furthermore, in both primary RPE cells isolated from pig retinas and the human ARPE19 cell line, application of a variety of S1R agonists enhanced cellular viability upon toxicity triggered by paraquat, an inhibitor of mitochondrial respiration. In contrast, an antagonist of S2R, CM398, which is highly selective for S2R versus S1R (Ki = 0.43 nM and 560 nM, respectively) protected cell viability in the presence of paraquat. To further define the specific role of S2R, we engineered a S1R knockout single-clone ARPE19 cell line using the CRISPR/Cas9 technology. No significant difference was observed in the protective effect of CM398 between wild type and S1R knockout cells, suggesting that the effect of CM398 was indeed mediated by S2R. Further supporting the S2R-mediated function, PB28, a S2R agonist, reduced cell viability in S1R knockout ARPE19 cells. In light of the critical importance of the RPE in maintaining a healthy retina, S1R agonists and S2R antagonists should be further evaluated for their potential in ameliorating age-related macular degeneration.

Disclosures: **T.A. Mavlyutov:** None. **A. Yao:** None. **U. Chu:** None. **M. Chu:** None. **L. Zhao:** None. **H. Yang:** None. **C.R. McCurdy:** None. **A.E. Ruoho:** None. **L. Guo:** None.

Poster

058. Retina: Photoreceptors

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Topic: D.04. Vision

Support: MOST-103-2628-B-002-001-MY3

Title: Light modulates body metabolism through melanopsin photodetection system

Authors: *C.-C. LEE, Y.-F. ZOU, S.-K. CHEN;
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Abstract: Ambient light signal has a profound impact on animal's physiological function. In 20 th century, human developed the additional light source, electricity light, lighted up the night time, and it has become a commonplace in human society. Recent studies in human suggested that exposure to the artificial light during nighttime (LAN) is strongly associated with overweight and metabolic diseases. In addition, studies in mice supported that the LAN exposure could cause those metabolic phenotypes without changing the total caloric intake; however, the percentage of daytime food intake was increased under the LAN condition. Whether light alone could affect the metabolism phenotypes remains unanswered. Moreover, the detailed mechanism of how light modulates energy metabolism stays unclear. Recently, intrinsically photosensitive retina ganglion cells (ipRGCs), which expressed novel photo pigments melanopsin, were known to transmit the light signal to control the non-image forming physiological function. Thus, we suspect that whether the melanopsin pathway is involved in the LAN effect on metabolism. Thus, we exposed the mice to light at night (~25 lux) with restricted feeding during the night time and examine their metabolic phenotypes. Here we reported that exposure to dim light during night time cause significant weight gain in mice and glucose intolerance; however, when the melanopsin protein was eliminated from the mice, both the weight gain and glucose intolerance were rescued. Moreover, in Brn3b positive ipRGC elimination mice (preserved circuitry to SCN), we found significant weight gain with normal glucose tolerance. Our data supports the melanopsin light detection signaling pathway and ipRGC circuitry are required in the direct light impact on energy metabolism in mice.

Disclosures: **C. Lee:** None. **Y. Zou:** None. **S. Chen:** None.

Poster

058. Retina: Photoreceptors

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A.B.G is an MRC-DTA Clinical Neuroscience PhD student

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R.A.P is a Royal Society University Research Fellow

Title: Targeted modulation of Crb1 protein within the recipient retina to improve photoreceptor transplantation efficiency

Authors: *A. B. GRACA, A. GEORGIADIS, C. HIPPERT, P. V. WALDRON, A. J. SMITH, R. R. ALI, R. A. PEARSON;
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Abstract: Purpose: Cell transplantation is a potential therapeutic strategy for the irreversible loss of photoreceptors (PRs). We, and others, have shown that immature PRs, derived either from neonatal donors or from stem cells, are able to functionally integrate into wild-type (wt) and diseased retinae. Despite these encouraging results, the number of donor cells that integrate within the recipient retina is small; hence it is essential to identify the barriers that impede donor cell integration and to devise strategies to remove them. We have previously identified the integrity of the outer limiting membrane (OLM) as a key determinant for transplantation outcome. The OLM is a series of adherens junctional protein complexes such as zona occludins (zo-1) and Crumbs 1 (Crb1), that form between Müller glia (MG) and PRs. Molecular disruption of zo-1 using siRNA enhances PR integration in wt recipients, but it also has detrimental effects on the neighbouring RPE. Since in the retina, Crb1 is restricted to the OLM, and the Crb1rd8/rd8 mouse model supports greater levels of integration compared to wt recipients, Crb1 might represent a better option for targeted OLM disruption in order to increase transplantation efficiency. Methods: Target short hairpins (sh) were sub cloned into the mU6 plasmid and tested

for efficiency. The most effective (sequence: 5'-GGAAGTGGATGAATGTGTTTCTGAT-3'; 80% knockdown) was cloned into the AAV pD10.CBA/RFP backbone before being packaged into the recombinant AAVShH10.Y445F vector, referred to herein as AAVShH10.Y444F-shCrb1. A non-targeting hairpin (sequence: 5'-GATCGGACACTCCTCATAA-3'; AAVShH10.Y444F-shControl) was used as a control. Both vectors were tested *in vitro* using MG cell cultures and then injected intravitreally into retinae of wt animals at postnatal day 8 and 18. Contralateral eyes received AAVShH10.Y444F-shControl. Eyes were harvested 3 weeks later and assessed by qRT-PCR and immunohistochemistry. Results: Administration of AAVShH10.Y444F-shCrb1 resulted in a robust reduction of Crb1 in MG cultures and in wt eyes. Histological assessments and immunohistochemistry show a disruption in OLM integrity in regions of viral transduction. No OLM disruption was observed in the eyes injected with control vector. Conclusions: There is a strong correlation between the integrity of OLM and transplanted PR integration. We report efficient knockdown of Crb1 in the retina using AAVShH10.Y444F-shCrb1, which leads to a loss of OLM integrity. Now we are investigating whether AAVShH10.Y444F-shCrb1 vector enables better transplanted PR migration and/or integration.

Disclosures: **A.B. Graca:** None. **A. Georgiadis:** None. **C. Hippert:** None. **P.V. Waldron:** None. **A.J. Smith:** None. **R.R. Ali:** None. **R.A. Pearson:** None.

Poster

058. Retina: Photoreceptors

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Topic: D.04. Vision

Title: How visual asymmetry starts in pigeons - characterizing Melanopsin as a potential trigger

Authors: ***R. KLOSE**, F. STRÖCKENS, K. SPOIDA, S. HERLITZE, O. GÜNTÜRKÜN; Ruhr-University Bochum, Bochum, Germany

Abstract: Structural and functional asymmetries of the nervous system, like handedness in humans, are a common feature in vertebrates. However, how the development of lateralization during ontogenesis is triggered remains largely unknown. For the asymmetry of the visual system in pigeons, we have strong evidence for the involvement of the environmental factor light. An asymmetrical position of the pigeon embryo inside the egg results in a stronger right eye light stimulation before hatch. This stimulation induces the development of a lateralized visual system affecting visual-guided behavior in adult pigeons. On the contrary, dark incubated pigeons do not show this asymmetry. However, classic retinal photoreceptors like rods and

cones, which perceive light stimulation in adult animals, are not functionally developed before hatch. This leads to the question, how an early asymmetrical light stimulation of the retina can induce visual lateralization. It has been shown that a third class of photoreceptive molecules occurs in retinal ganglion cells of other species. Since retinal ganglion cells are present at early developmental stages and already possess functional connections to primary visual areas before hatch, retinal ganglion cell bound photoreceptors could serve as a potential trigger for visual lateralization. Here we addressed the question if one of these photosensitive molecules, Melanopsin, could be such a trigger for asymmetry formation. To proof the particular role of Melanopsin during lateralization in pigeons, we were already able to sequence two isoforms of the Melanopsin gene in pigeons as well as demonstrating the expression of Melanopsin in the retina during all critical developmental stages (embryonic day 16 + post-hatch day 2). Now, to further support our hypothesis, we tried to discover if Melanopsin-positive cells can be activated by light. By performing an *in vitro* calcium assay using a HEK cell model, we discovered that both Melanopsin isoforms need the cofactor 9-cis retinal as reported in the literature for other Melanopsin genes. However, isoforms showed different light sensitivities resulting in higher calcium signals evoked by the long isoform. Our results provide for the first time a closer characterization of Melanopsin in pigeons. We currently plan to quantify the expression of both Melanopsin isoforms in the retina over three developmental stages (embryo, hatchling, adult). All together this could deliver a considerable support to our theory that Melanopsin plays a key role in the induction of lateralization in pigeons.

Disclosures: **R. Klose:** None. **F. Ströckens:** None. **K. Spoida:** None. **S. Herlitze:** None. **O. Güntürkün:** None.

Poster

058. Retina: Photoreceptors

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Title: Müller cells as light guiding fibers in caiman

Authors: *A. ZAYAS-SANTIAGO[1], A. SAVVINOV[4], S. AGTE[5], Y. RIVERA[1], J. BENEDIKT[1], L. A. CUBANO[2], A. REICHENBACH[5], S. N. SKATCHKOV[3]; [1]Physiol., [2]Anat., [3]Physiol. & Biochem., Univ. Central Del Caribe, Bayamon, PR; [4]Physical Sci., Univ. de Puerto Rico, Recinto de Río Piedras, Río Piedras, PR; [5]Paul Flechsig Inst. of Brain Res. Fac. of Med., Univ. of Leipzig, Leipzig, Germany

Abstract: This study highlights the newly discovered function of glia: light propagation via nano-fibers (Müller glial stalks) in the inverted retina. In the peripheral retina, photons of light should pass through all sorts of light scattering elements, such as cell bodies, synapses, axons, dendrites, and blood vessels, before reaching the photoreceptors and starting the visual process. This means that without special light guiding objects, vision will be restricted. Müller glial cells, which have a cylindrical fiber-like shape, act as light-guiding optical fibers that contribute to light propagation from the inner retinal surface directly to the outer segments of photoreceptors. Here we show that glial Müller cells in caiman (Caiman crocodilus fuscus): (i) cross all light scattering objects in the retina, (ii) are aligned in the direction of the light's path, (iii) have a higher refractive index than their surrounding elements, that help to trap and to hold the light, and (iv) are tightly connected to the photoreceptor cells at the end of the light pathway. We demonstrate using immunocytochemistry the presence of specific glial proteins such as: GFAP, S100B, and vimentin, which can form intracellular nano-fiber structures. Finally, we show that very thin Müller glial cells in caiman can conduct light signals. A living caiman retinal whole-mount was placed in a supporting chamber on the confocal microscope stage with the endfeet oriented down towards the light coming from the laser. This light was focused to a single endfoot by a 10X objective. The image was captured using another 40X objective at the cone-rod outer segment layer. A double cone photoreceptor (DCP) was observed illuminated, meaning that a single Müller cell endfoot projects light to a single DCP. Caiman Müller cells show both single and multiple endfeet; the cells with multiple endfeet could maximize the number of photons collected and projected to a DCP. Therefore, we suggest that this type of optic fiber alignment can be used as a novel type of "amplifying array" that increases the amount of photons absorbed by a photoreceptor cell. (Authors A.Z-S., A.S. & S.A. contributed equally to this work.)

Disclosures: A. Zayas-Santiago: None. **A. Savvinov:** None. **S. Agte:** None. **Y. Rivera:** None. **J. Benedikt:** None. **L.A. Cubano:** None. **A. Reichenbach:** None. **S.N. Skatchkov:** None.

Poster

058. Retina: Photoreceptors

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Topic: D.04. Vision

Support: 1T32EY22303-1A1

Title: Phthalazinone pyrazole enhances photoreceptor differentiation and survival: implications for a multitarget mechanism of action

Authors: *J. A. FULLER[1], D. HELM[2], I. RUCZINSKI[3], K. J. WAHLIN[1], C. BERLINICKE[1], R. MACARTHUR[4], P. K. DRANCHAK[4], M. MATTHES[5], M. M. LAVAIL[6], B. KUSTER[2], J. INGLESE[4], D. J. ZACK[1];
[1]Ophthalmology, Johns Hopkins Sch. Med., Baltimore, MD; [2]Technische Univ. Muenchen, Freising, Germany; [3]Johns Hopkins Sch. of Publ. Hlth., Baltimore, MD; [4]NIH Natl. Ctr. for Advancing Translational Sci., Rockville, MD; [5]UCSF, Rockville, CA; [6]UCSF, San Francisco, CA

Abstract: Retinal degenerations are a heterogeneous group of diseases in which there is a progressive loss of photoreceptors. There are currently no FDA approved therapies to slow photoreceptor loss, and as such, identification of protective compounds has been a long-sought therapeutic goal. Recent advances in stem cell biology has made photoreceptor transplantation an increasingly realistic possibility. However, current methodologies for photoreceptor differentiation and maturation are slow and relatively inefficient. In addition, current photoreceptor transplantation methodologies in animal models show a low integration and survival rate. In an effort to identify novel small molecules that both enhance survival and promote photoreceptor differentiation, we have microscaled (1536 well) assays utilizing cultured primary retinal neurons, and are able to culture in this format for up to 14 days. From a focused library screen of 107 small molecules utilizing cells from a GFP knock-in reporter mouse (Rhodopsin-EGFP), we identified phthalazinone pyrazole (4-[(5-methyl-1H-pyrazol-3-yl)amino]-2H-phenyl-1-phthalazinone, PHPZ), originally identified as a selective Aurora-A inhibitor, that increases the number of photoreceptors in culture in a concentration-dependent format. A qRT-PCR array of 95 photoreceptor-associated genes was performed and we found that the molecule upregulates several rod-specific genes including Rhodopsin, NR2E3, and NRL. Viability assays (CellTiterGlo and Calcein/Ethidium Homodimer) utilizing immunopan-purified photoreceptors shows that the PHPZ specifically enhances cultured photoreceptor survival in a phthalazinone-dependent fashion. Rhodopsin S-334ter rats, a retinitis pigmentosa model, were injected intravitreally at postnatal day 9 with 1 μL of a .15, .075, or .0075 mg/mL solution of PHPZ and sacrificed 10 days later. PHPZ treatment (.15 or .075 mg/mL) significantly increased the number of surviving photoreceptors. A chemical proteomic methodology utilizing Kinobeads™ was used to profile the binding interactions of PHPZ with kinases in retina lysate. Several sub-micromolar interactions with kinases were identified, including GSK3α/β (Kd: 14,

12 nM respectively), DLK/MAP3K12 (122 nM), and STK4/MST-1 (602 nM), which have been proposed to be involved in photoreceptor survival and/or differentiation. These results suggest that a phenotypic primary cell-based screen used in conjunction with chemical proteomic methodologies can be used to identify novel preclinical candidates as well as pharmacological networks that favor enhancement of photoreceptor survival and maturation.

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Poster

058. Retina: Photoreceptors

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Support: NIH R01EY017452

NIH R21 EY023339

Title: A new role for AMP-activated protein kinase (AMPK) in the circadian regulation of L-type voltage-gated calcium channels (L-VGCCs) in retinal photoreceptors

Authors: C. C.-Y. HUANG, L. SHI, C.-H. LIN, A. J. KIM, M. L. KO, *G. Y.-P. KO;
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Abstract: The AMP-activated protein kinase (AMPK) is a cellular energy sensor, which is activated when the intracellular ATP production decreases. The activities of AMPK display circadian rhythms in various organs and tissues, indicating that AMPK is part of the circadian regulation for cellular metabolism in these tissues. Disruption of AMPK genetically alters the circadian behavior rhythms in rodents. In vertebrate retina, the circadian clocks regulate many

aspects of retinal function and physiology, including light/dark adaption. We found that the overall retinal ATP content displayed a diurnal rhythm, which was nearly anti-phase to the diurnal and circadian rhythms of AMPK phosphorylation/activation. In addition, AMPK was involved in the circadian phase-dependent regulation of L-type voltage-gated calcium channels (L-VGCCs). In cone photoreceptors, the L-VGCCs are essential for sustained neurotransmitter release and crucial for retinal light sensitivities. The protein expression and currents of L-VGCCs are higher at night and lower during the mid-day. The activation of AMPK dampened the L-VGCC currents at night with a corresponding decrease in protein expression of the L-VGCCα1 pore-forming subunit, while inhibition of AMPK increased the L-VGCC current during the day. The AMPK appeared to be upstream of extracellular-signal-regulated kinase (ERK) and mammalian/mechanistic target of rapamycin complex 1 (mTORC1) in regulating the circadian rhythm of L-VGCCs. Hence, we demonstrated that in addition to serving as a cellular energy censor, AMPK integrates into the cell signaling network to regulate the circadian rhythm of photoreceptor physiology. Support: This work was supported by R01EY017452 and R21 EY023339 from the National Eye Institute of the National Institutes of Health to GK.

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Poster

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Title: Ursodeoxycholic acid attenuates endoplasmic reticulum stress-related pericyte loss in diabetic retinopathy of streptozotocin-treated mice

Authors: *J. CHOI[1], Y.-R. CHUNG[2], Y. HAN[1], J.-Y. KOH[3], Y. YOON[4];
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Ajou Univ. Sch. of Med., Suwon, Korea, Republic of; [3]Neurol., [4]Ophthalmology, Asan Med. Center, Univ. of Ulsan Col. of Med., SEOUL, Korea, Republic of

Abstract: Loss of pericytes, an early hallmark of diabetic retinopathy (DR), results in the breakdown of blood-retinal barrier. Endoplasmic reticulum (ER) stress is thought to be involved in this process. This study is to test ursodeoxycholic acid (UDCA) that is known to ameliorate ER stress on pericyte loss in DR of streptozotocin (STZ)-induced diabetic mice. To assess the extent of DR, the integrity of retinal vessels and density of retinal capillaries in STZ-induced diabetic mice were measured. In addition, the induction of ER stress and unfolded protein response (UPR) were assessed in diabetic mice and human retinal pericytes exposed to advanced glycation end products (AGE) or modified low-density lipoprotein (mLDL). Leakage of fluorescein dye on angiography and decreased density of retinal capillaries were improved in UDCA-treated diabetic mice compared to non-treated diabetic group. Among the UPR markers, the expressions of markers involved in the PERK pathway were increased in STZ-induced diabetic mice, as well as AGE or mLDL-exposed retinal pericytes in culture. UDCA treatment blunted the increase of UPR markers in these cases. Administration of UDCA also inhibited mLDL- and AGE-induced death of cultured pericytes. UDCA attenuated UPR in retinal cells of DR models, *in vitro* and *in vivo*. Likely as a result, UDCA ameliorated vascular integrity and pericyte loss in the retina of STZ-induced diabetic mice. This suggests that UDCA, putatively a chemical chaperone that alleviates ER stress, may prove effective in protecting against diabetic retinopathy.

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Poster

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Title: Generation of Sox4 mutant zebrafish using CRISPR/Cas9

Authors: ***W. WEN**[1], L. PILLAI-KASTOORI[2], S. WILSON[2], A. KRISHNA[2], A. MORRIS[2]; [1]Univ. of Kentucky, Lexington, ; [2]Univ. of Kentucky, Lexington, KY

Abstract: Objective: Sox4 is a member of the group C SRY-box containing transcription factors. It promotes differentiation of multiple neuronal lineages and is required for neural progenitor survival. Zebrafish have two co-orthologs of the mammalian sox4: sox4a and sox4b. They are strongly expressed in the developing zebrafish eye. Morpholino-mediated sox4a/b knockdown in zebrafish leads to elevation in Hh activity, causing ocular coloboma and reduced number of mature rod photoreceptors. The effectiveness of morpholino is reduced by 4 days post fertilization (dpf) in zebrafish due to rapid cell proliferation. In order to study the role of sox4 in ocular development at later developmental stages as well as during adult retina regeneration, we generated sox4a and sox4b mutant zebrafish lines using CRISPR/Cas9. Methods: Sox4a and sox4b CRISPR target sites were identified and the corresponding single strand guide RNA (sgRNA) oligos were designed using the ZiFiT online software. sgRNAs and the Cas9 mRNA were synthesized according to the published protocol, and were microinjected into fertilized zebrafish embryos (F0) at the one-cell stage. F0s were raised to adulthood and screened for germline transmission by outcrossing with wild-type (WT). F1s were screened for inheritance of sox4 mutant allele using high resolution melting analysis (HRMA) and sequencing. F1 heterozygous mutants were raised to adulthood and outcrossed with WT to generate F2s. F2s were incrossed to generate F3s, of which 25% were expected to be homozygous mutant for sox4. Individuals with ocular coloboma were fixed with 4% paraformaldehyde at 5 dpf and cryosectioned for immunohistochemistry. Results: Founders carrying frameshift mutations in sox4 were successfully generated at the sox4aC1, sox4aC2, and sox4bC2 loci (C1 targets upstream of the HMG domain, C2 targets upstream of the transactivation domain). Mutant alleles were stably passed to the F1-F3 generations. Sox4aC1, sox4aC2, and sox4bC2 homozygous mutant individuals were identified in the F3 generation. Occasionally, ocular coloboma was observed in either heterozygous or homozygous sox4bC2 mutant embryos. However, the penetrance of ocular coloboma was less than 2%. Conclusion: Sox4a and sox4b mutant zebrafish were generated by CRISPR/Cas9. Perhaps due to functional redundancy between sox4a and sox4b, the ocular coloboma phenotype was only observed in a small proportion of sox4b mutants. Future plans include crossing the sox4a and sox4b mutants to generate sox4a/b double mutants, and characterizing the mutant alleles *in vivo* to verify that they cause a loss of function in Sox4.

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Poster

058. Retina: Photoreceptors

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HHMI

Title: Identification and characterization of intrinsically photosensitive retinal ganglion cells in the common snapping turtle, Chelydra serpentina

Authors: C. R. HELLER[1], S. C. BEHLING[2], E. N. SUTTER[2], A. E. TURNER[1], E. A. ULANDAY[2], *J. A. DEMAS[3];
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Abstract: In freshwater turtles, the navigation of hatchlings towards water is crucial to survival. Previous studies have shown that hatchling snapping turtles navigate using light cues. The retinal substrate for this behavior is not well understood. However, in neonatal mice, which lack functional rods and cones, phototactic behavior is mediated by intrinsically photosensitive retinal ganglion cells (ipRGCs). These findings raise two questions: are ipRGCs present in turtle retinas? If so, do ipRGCs mediate hatchling navigation? Using a multi-electrode array, we recorded *in vitro* from the retinas of hatchling and juvenile snapping turtles (Chelydra serpentina).To identify ipRGCs, we compared RGC responses to 60 s light flashes (480 nm, 5e14 photons/cm^2/s) before and after the addition of a neurotransmitter antagonist cocktail (CNQX, D-APV, APB, hexamethonium bromide, atropine, bicuculline, CGP52432, strychnine). In both hatchlings and juveniles, most RGCs stopped responding to light after adding the cocktail. However, a subset of RGCs continued to increase their firing rate in response to the onset of the light flashes. These responses resembled the intrinsic light responses previously characterized in mammalian ipRGCs, including a relatively sustained, long latency (>1s) response. We quantified the absolute sensitivity of these putative ipRGCs to varying irradiances of 480 nm light (1e13 to 1e16 photons/cm^2/s). With increasing irradiance, the amplitude of ipRGC responses increased, while response latencies decreased. We physiologically identified two subclasses of ipRGCs with different absolute sensitivities and response latencies to light. In mammalian ipRGCs, melanopsin mediates this intrinsic response. PCR experiments demonstrated that the turtle retina also expresses melanopsin, suggesting that melanopsin could mediate the intrinsic light response in turtle RGCs. Indeed, the addition of opsinamide, a melanopsin antagonist, significantly attenuated the light responses of turtle ipRGCs. Future work will include characterization of the action spectrum of the turtle ipRGC light response to test its consistency with a melanopsin-based phototransduction mechanism. Additionally, we have

created a behavioral assay to determine whether these cells play a role in hatchling navigation. In a Y-maze, turtles demonstrated robust positive phototactic behavior. We will administer intraocular injections of the neurotransmitter antagonist cocktail to determine whether ipRGCs are sufficient to mediate this phototactic behavior.

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Poster

058. Retina: Photoreceptors

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Topic: D.04. Vision

Title: Transcriptome analysis of ipRGCs with next-generation sequence

Authors: *N.-F. LIOU[1], H. WANG[2], S. CHEN[1];
[1]Life Sci., [2]Sec Dept. One Life Sci. , Natl. Taiwan Univ., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Intrinsic photosensitive retina ganglion cell (ipRGC) is a special type of retina ganglion cell, which can express melanopsin and sense the light signal. As we know from previous studies, ipRGC is involved in circadian photo-entrainment, pupil reflex and sleep. And ipRGC could be separated into at least five types according to their neurites morphology and cell body size. Moreover, evidence suggests that different types of ipRGC could project to distinct brain area to influence specific physiological functions. However, the differentiation cue or specific marker for individual type of ipRGC remains unclear. Using single cell RNA sequencing technic, we performed transcriptome analysis between ipRGC and regular RGC to identify whether ipRGCs express any specific gene that is important for their specification.

Disclosures: N. Liou: None. **H. Wang:** None. **S. Chen:** None.

Poster

058. Retina: Photoreceptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 58.21/J32

Topic: D.04. Vision

Support: Canadian Institutes of Health Research

Natural Sciences and Engineering Council of Canada

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Title: Molecular and functional characterization of opsins and TRP channels in compound eyes of the cockroach, Periplaneta americana

Authors: *A. S. FRENCH[1], P. H. TORKKELI[1], S. MEISNER[1], H. LIU[1], E.-V. IMMONEN[2], R. FROLOV[2], M. WECKSTROM[2];
[1]Dept Physiol & Biophysics, Dalhousie Univ., Halifax, NS, Canada; [2]Res. Ctr. for Mol. Materials, Univ. of Oulu, Oulu, Finland

Abstract: Detailed mechanisms of insect phototransduction have been explored in a limited number of species, and particularly in the dipteran *Drosophila* melanogaster, where rhodopsin photoisomerization activates a Gq-protein, initiating a cascade leading to activation of IP3 and diacylglycerol. This cascade opens transient receptor potential (dTRP) and TRP-like (dTRPL) ion channels via uncertain mechanisms. The relative contributions of dTRP and dTRPL channels to total light-activated currents under physiological conditions are not yet certain, but dTRP channels probably dominate. Insects utilize a wide range of visual environments and lifestyles that impose varying constraints on visual function, with resultant changes in phototransduction machinery. For example, *Drosophila* fly rapidly under mainly bright light conditions, but cockroaches occupy a primarily terrestrial, nocturnal or crepuscular environment that requires greater visual sensitivity but less rapid responses. Electrophysiological evidence suggests that cockroach phototransduction makes major contributions to this environmental adaptation, including a greater role for the homologous pTRPL rather than pTRP channels. We created a transcriptome of Periplaneta compound eye retina and assembled mRNA sequences for three opsins and two TRP channels. Relative mRNA abundances in normal retina were estimated from representation in the transcriptome and by quantitative PCR (qPCR). Phylogenetic analysis indicated that two opsins (pGO1 and pGO2) are green absorbing and one (pUVO) is UV absorbing, with pGO1 mRNA being 10-100 times more abundant than the others. The two TRP genes were similarly identified as pTRP and pTRPL, with pTRPL mRNA being 10 times more abundant than pTRP. We developed protocols for *in vivo* RNA interference (RNAi) based gene silencing to suppress translation of these genes by injecting long (500-700 bp) double stranded RNA (dsRNA) into the cockroach head hemolymph. Gene suppression in RNAi treated animals was measured by qPCR. Photoreceptor function was examined by electroretinograms (ERG) of intact eyes, and by whole-cell patch clamp recordings of single photoreceptors in dissociated ommatidia. Injection of dsRNA corresponding to the major green opsin, pGO1 caused almost

complete elimination of phototransduction within seven days. RNAi of the TRP and pTRPL genes proceeded more slowly, taking up to 21 days, and reduction of pTRPL had a more profound effect on light response, supporting a more important role for pTRPL than pTRP in Periplaneta phototransduction.

Disclosures: **A.S. French:** None. **P.H. Torkkeli:** None. **S. Meisner:** None. **H. Liu:** None. **E. Immonen:** None. **R. Frolov:** None. **M. Weckstrom:** None.

Poster

058. Retina: Photoreceptors

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 58.22/J33

Topic: D.04. Vision

Support: RO1 EY012141

Title: Mammalian cone photoreceptors are capable of extraordinarily rapid release and maintain a large number of ribbon-tethered vesicles in reserve

Authors: *C. P. GRABNER[1], S. H. DEVRIES[2];
[1]Dept. of Ophthalmology, Chad Grabner, Chicago, IL; [2]Physiol. and Ophthalmology, Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: One of the first processing steps in vision occurs when the steady changes in membrane voltage produced in a cone photoreceptor by light are transmitted to postsynaptic neurons at ribbon synapses that can mediate both transient and sustained release. Cone bipolar cells further transform the cone output in a type-selective manner through the expression of different glutamate receptors and by the position and number of their contacts at the cone terminal. In order to understand the complex transformations that occur at the cone synapse, we began by directly determining the properties of release by performing membrane capacitance measurements in the ground squirrel, which has a cone dominant retina. In particular, we wanted to understand the relationship between ribbon recovery from depression and the recovery of postsynaptic Off bipolar cell glutamate receptors from desensitization. Peak release was obtained during steps to -10mV, while steps to more positive potentials showed incremental reductions as the Nernstian drive for Ca^{2+} decreased. For steps to voltages more negative than -10 mV, release was delayed, consistent with the activation kinetics of Ca^{2+} channels being voltage-dependent. To more carefully explore the kinetic phases of release, pulse duration was varied while V_m was stepped to -10 mV. This approach revealed two rapid kinetic phases for release with τ's of 0.5

and 7 ms, and assuming 20 ribbons/cone terminal, each phase involved 13 and 7 vesicles/ribbon, respectively, which equates to instantaneous release rates of ~26,000 and 600 vesicles/s/ribbon. Using brief 1 ms steps in a paired-pulse recovery protocol, or a train of step depolarizations, the primed vesicles refilled at a rate of 13 vesicles/s/ribbon, equivalent to a recovery $\tau \sim 1$ s. A distinct late phase of release emerged with prolonged step depolarizations and had a $\tau \sim 250$ ms and an estimated release rate of 40 vesicles/s/ribbon. Electron microscopy was used to estimate the packing density of vesicles at cone ribbons, and gave a density of ~ 70%, yielding 25 docked vesicles at the ribbon base and a total of 95 vesicles/ribbon. Taken together, the extraordinarily rapid initial release phase emptied most of the docked vesicles within 1 ms, and all docked vesicles were released within 30 ms. The subsequent re-priming of docked vesicles is relatively slow when probed with 1 ms steps, ~ 1 s, and only a continual 1 s step invokes the release of an equivalence of all ribbon tethered vesicles. Thus, ground squirrel cones recover at a rate slower than the postsynaptic recovery rate in some Off cone bipolar cell types (cb2: ~150 ms) and similar to or faster than others (cb3: ~ 800-900 ms).

Disclosures: C.P. Grabner: None. **S.H. DeVries:** None.

Poster

058. Retina: Photoreceptors

Location: Hall A

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Topic: D.04. Vision

Support: NIH Grant EY012141

Title: A threshold non-linearity at the mammalian cone photoreceptor basal synapse

Authors: *S. H. DEVRIES;
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Abstract: Purpose: The goal of this study is to investigate the input-output properties of the mammalian cone photoreceptor basal synapse. Cones make two types of synaptic contacts with bipolar cells, invaginating and basal. There are ~20 invaginations per terminal and each is marked at its apex by a synaptic ribbon. Basal contacts involve close, ~20 nm, appositions between the membranes of a cone and Off bipolar cells at the base of the terminal. Receptors at basal contacts are thought to receive transmitter from distant ribbons (200-500 nm). Transmission at such distances is normally associated with spillover, and it is unclear whether basal contacts can sense and respond to the release of a single vesicle from a ribbon or whether

they respond only to multivesicular events. Methods: I used dual cell voltage clamp to record from pairs of synaptically connected cones and Off bipolar cells (types cb1 and cb3) in slices from the ground squirrel retina. In a typical experiment, a train of 1 ms pulse depolarizations from -70 mV were applied to a cone and epsc responses were recorded in both the presynaptic cone (via the glutamate transporter) and postsynaptic bipolar cell. With small depolarizations, the cone events fluctuate and have quantal-type properties. Results: For a train of cone depolarizations, I plotted the amplitude of the fluctuating responses in the cone versus the bipolar cell. The strength of the cone stimulus was then adjusted to obtain an extended plot of responses. Cone responses were converted from pA to quanta based on a fluctuation analysis, and a linear range was established. Bipolar cell responses were not normalized. The scatter plots for cb1a cells were striking in that they typically had a range of 0-10 cone vesicles released during which no postsynaptic response could be detected. Only at stronger depolarizations were responses evident. An analysis of spontaneous release at the cone to cb1a cell synapse also consistently showed a failure to respond at the quantal level, but occasional responses to multivesicular events were observed. Cb3 cells demonstrated a similar although less marked response non-linearity. Conclusions: The striking result for the cb1a cell, and to a lesser extent for the cb3 cell types, is the failure to reliably detect single quantal events. One possible mechanism for this failure is the distance between the ribbon release sites and the basal contacts of the recorded bipolar cells.

Disclosures: S.H. DeVries: None.

Poster

058. Retina: Photoreceptors

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Topic: D.04. Vision

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ISPB research grant

Title: Integration of luminance, chromatic signals and melanopsin activation in human ipRGC processing

Authors: *P. BARRIONUEVO, D. CAO;
Ophthalmology and Visual Sci., Univ. of Illinois At Chicago, Chicago, IL

Abstract: Intrinsically photosensitive retinal ganglion cells (ipRGCs) express melanopsin, a photopigment that can respond to light directly. In addition, these cells receive afferent inputs from rods and cones. Primate ipRGCs display S-OFF, L+M ON, and melanopsin ON response property. We investigated the integration of luminance and chromatic signals with melanopsin activation in ipRGCs processing by measuring human pupillary flickering responses. Using a lab-developed five primary photostimulator that can control the excitations of rods (R), three types of cones (S, M, L) and melanopsin-mediated ipRGCs (I) independently, we measured pupillary responses to sinusoidal stimuli that modulated rod, cone and melanopsin excitations in isolation or together but with relative phase varied. We used three types of cone stimuli, including cone luminance (LMS), equiluminant L vs. M [L/(L+M)], and S-cone (S) modulations. The temporal frequency was 1 Hz at 200 Td and 2000 Td. Consensual pupil recordings were obtained from the left eye with an EyelinkII eyetracker (Sampling rate 250Hz, spatial resolution<0.01mm). The results showed that S-cone-driven pupillary responses were out of phase with respect to I, LMS and R responses, with LMS producing the largest responses but S-cones producing the weakest responses among the isolated conditions. Melanopsin-driven pupillary responses were approximately linear with melanopsin contrast (2% to 18%), maintaining phase near 0°. S-cone-driven contrast response (10% to 85%), however, saturated at ~50%; with the response phase of 180° for the entire contrast range tested. Variation of I phases with respect to LMS, S, L/(L+M) and R signals could be described by a vectorial summation model of the weighted isolated responses, suggesting that melanopsin activation is linearly combined in ipRGCs with cone luminance and chromatic signals to control phasic pupil responses. Melanopsin contribution was small in combination with LMS but dominant in combination with R, S, and L/(L+M). Because S-cone contribution is very weak with respect to other inputs, out-of-phase S-cone input is usually masked by other photoreceptor inputs. Our results suggest that ipRGCs receive signals from retinal pathways involved in luminance and chromatic processing. The luminance and chromatic signals are integrated linearly with melanopsin signal in ipRGCs. The weightings from the vector summation models indicated that ipRGCs received strong synaptic inputs from diffuse bipolars in the magnocellular pathway for luminance processing but weak inputs from bipolars or amacrines in the parvocellular and koniocellular pathways for chromatic processing.

Disclosures: **P. Barrionuevo:** None. **D. Cao:** None.

Poster

059. Population Coding in Striate Cortex

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 59.01/J36

Topic: D.04. Vision

Title: Turtle visual cortex studied by combining wide-field calcium imaging and electrophysiology

Authors: *M. A. LAUTERBACH, M. HEMBERGER, M. SHEIN-IDELSON, G. LAURENT; Dept. of Neural Systems, Max Planck Inst. For Brain Res., Frankfurt, Germany

Abstract: Reptilian cerebral cortex contains 3 layers, similar to hippocampal and piriform cortices of mammals. Pyramidal cells are found only in layer 2, the only real soma layer, which is thought to be equivalent to (output) layers 5/6 of mammalian isocortex [1]. Turtle cortex offers well-known experimental advantages: it is resistant to anoxia and operates at room temperature. Cortical slabs can be cut in orientations that minimize lesions and keep inter-neuronal connections intact. The entire brain can even be extracted and kept *in vitro* for several days. Finally, the sparse neuronal activity observed there is well adapted to calcium imaging, where each action potential can be observed as a prolonged signal. Processing of visual information in turtle cerebral cortex is not understood, and clear single-neuron receptive field properties have yet to be discovered there [2]. Our lab combines *in vivo* and more reduced approaches to understand the functions and operations carried out by this simple cortex. Here we show network analysis of activity in the visual cortex of turtles (Trachemys scripta/Chrysemys picta) using a combination of electrophysiology and calcium imaging. Advanced image processing enables calcium imaging in wide-field mode (single-photon excitation) and thus the fast monitoring of large fields of view in combination with electrophysiological methods. Sensitivity is sufficient to resolve sub-threshold signals in cell bodies and calcium signals elicited by single action potentials can be detected even in dendrites. Using AM-dyes, hundreds of neurons can be stained and monitored very easily. A depth penetration of more than 100 µm is obtained, sufficient to image cortical layer 2 from the ventricular surface in an *ex vivo* preparation. By combining imaging with patch-clamp recordings of one or a few interneurons, the distributed and coordinated effects of single interneurons on regularly spiking neurons can be analyzed. By combining imaging with multi-electrode array (MEA) recordings, electrical signals from hundreds to thousands of spiking neurons can be observed. After spike sorting (i.e. the assignment of the electrical signals to discrete neurons) electrical activity can be correlated with sub- and supra- spike-threshold calcium signals enabling a broad ensemble view of stimulus-evoked and spontaneous activity in this system. [1] Aboitiz, F; Zamorano, F: Neural progenitors, patterning and ecology in neocortical origins. Front Neuroanat 2013, 7:38. [2] Fournier, J; Christian, M M; Laurent, G: Looking for the roots of cortical sensory computation in three-layered cortices. Current Opin Neurobiol 2014, 31:119

Disclosures: **M.A. Lauterbach:** None. **M. Hemberger:** None. **M. Shein-Idelson:** None. **G. Laurent:** None.

Poster

059. Population Coding in Striate Cortex

Location: Hall A

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Topic: D.04. Vision

Support: National Natural Science Foundation of China 31371111

the Hundred Talent Program of the Chinese Academy of Sciences

Title: Intra-areal patterns found in spontaneous cortical activity

Authors: ***H. XU**[1,2], P. LI[1], C. HAN[1,2], S. ZHU[1,2], Y. FANG[1,2], M. CHEN[1,2], J. HU[1,2], H. MA[1], Z. JI[1], C. FANG[1], H. D. LU[1];
[1]SKLCNL, Beijing Normal Univ., Beijing, China; [2]Inst. of Neuroscience, CAS, Shanghai, China

Abstract: Recent fMRI studies examining resting state functional connectivity (RSFC) have provided insight into the inter-areal functional networks in the human brain. However, due to the spatial resolution limit of fMRI, the existence and properties of intra-areal RSFC remain unclear. To explore the RSFC in a finer scale, we imaged intrinsic optical signals in anesthetized monkey visual cortex (V1 V2 and V4) without visual stimulation. In these spontaneous conditions, slow (<0.4Hz) hemodynamic fluctuations exhibited multi area-specific topographic patterns, most of which matched the known functional maps (e.g. ocular dominance, orientation, and color maps) obtained in visual stimulation conditions. Novel spontaneous patterns were also observed, mainly in area V4, and did not match any known functional maps in this area. Within one area, temporal fluctuations of spontaneous activity in orthogonal functional domains were negatively correlated, while fluctuations in the same type of domains in different visual areas were positively correlated. These findings demonstrate that slow ongoing optical signal contains fine-scale network information which is tightly related to the underlying anatomical and functional networks. Optical imaging of RSFC thus provides a new way in exploring intrinsic network properties, particularly useful for cortical areas that are difficult to be studied in regular stimulus or task paradigms.

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Poster

059. Population Coding in Striate Cortex

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DARPA contract W91NF-14-1-0269 (RY)

Title: Coordinated neocortical activity at cellular resolution during visual processing

Authors: *Z. MA[1], Y. KARIMIPANAH[1], J.-E. MILLER[2], R. YUSTE[2], R. WESSEL[1]; [1]Physics, Washington Univ. In St. Louis, Saint Louis, MO; [2]Dept. of Biol. Sci., Columbia Univ., New York, NY

Abstract: The highly interconnected nature of cerebral cortex supports the hypothesis that cortical function emerges from coordinated neural activity across scales of space and time. Testing this tantalizing coordination hypothesis ultimately requires recording neural activity at spatial scales from synapses, dendrites, neurons, microcircuits to brain regions and during sensory processing. Here we performed two-photon population calcium imaging of layer 2/3 neurons in primary visual cortex of awake and behaving mice during three conditions of visual stimulation: black screen, drifting grating, or natural movie. For each mouse and stimulus condition we obtained the inferred spike trains from some 100 closely-spaced neurons for several minutes. We analyzed the population of spike trains for each mouse and condition with respect to (i) the statistical properties of individual spike trains, (ii) the pairwise correlation of spike trains, and (iii) the coordination across neurons and time for all spike trains of a given data set. The analysis of the population of spike trains revealed three important features. First, spike trains showed a broad distribution of mean rates and highly irregular spiking. The latter resulted in a broad distribution of the coefficients of variation of the inter spike intervals with a population mean larger than one. Second, pairs of spike trains were weakly correlated resulting in a distribution of small values of cross correlation coefficients for all pairs. Third, neuronal avalanches, which are cascades of contiguous spikes within the population of imaged neurons, had power law size and duration distributions, while shuffled results didn't. Furthermore,

avalanche sizes and durations followed a scaling relation, which is an important fingerprint of a dynamical critical system. In addition, the statistical properties of the population spike trains were largely independent of the stimulus condition, thus indicating a dominant contribution from intracortical dynamics. Taken together, this collection of quantitative observations of cortical population activity during visual stimulation provides valuable constraints for future models of cortical dynamics and sensory processing.

Disclosures: Z. Ma: None. **Y. Karimipanah:** None. **J. Miller:** None. **R. Yuste:** None. **R. Wessel:** None.

Poster

059. Population Coding in Striate Cortex

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: D.04. Vision

Support: CRSNG Grant 6943-2010RGPIN

Title: Characterizing soloists and choristers in primary visual cortex

Authors: *L. BACHATENE[1], V. BHARMAURIA[1], S. CATTAN[1], N. CHANAURIA[1], J. ROUAT[2], S. MOLOTCHNIKOFF[1];
[1]Dept. of Biol. Sci., Univ. De Montréal, Montreal, QC, Canada; [2]Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: Sensory informations are computed in the cortex by networks of co-activated neurons forming functional ensembles. Visual processing in the cortex underlies several aspects of neuronal characteristics such as anatomical, electrophysiological and molecular. In particular, the coupling of spiking activity is an important indicator of the dynamic within a neuronal population. The activity of a neuron classified as a ‘soloist’ is distinct from the firing pattern of a so called ‘chorister’*. These two classes correlate differently their firing rate with the global populational activity*. In the present investigation, we sought to examine the relationship between the firing patterns (fast or regular spiking) of cortical neurons simultaneously recorded from the cat’s primary visual cortex and their tendency to act as a soloist or a chorister. We observed that fast-spiking neurons which are commonly attributed to putative inhibitory interneurons mainly correlate their firing with the neuronal population (choristers). On contrary, regular spiking neurons considered as putative excitatory pyramidal neurons exhibited weak coupling activity with their neighboring population (soloists). Major consequences may arise

from this research. Importantly, estimating the correlation of spike-trains of neurons with their neighboring assembly is a predictive indicator of their neuron-type. These results give new insights into visual coding by demonstrating how putative inhibitory interneurons frame the neuronal responses of principal cells within neuronal-assemblies. *M. Okun, N.A. Steinmetz, L. Cossell, M.F. Iacaruso, H. Ko, P. Bartho, T. Moore, S.B. Hofer, T.D. Mrsic-Flogel, M. Carandini, K.D. Harris, Diverse coupling of neurons to populations in sensory cortex, Nature (2015). Support CRSNG and FRQ-NT to SM

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Poster

059. Population Coding in Striate Cortex

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NSF CRCNS grant #1308159 (R.W.)

Title: Irregular spiking and broad bate distributions both arise as emergent properties of sparsely connected recurrent networks

Authors: *Y. KARIMIPANAH[1], Z. MA[2], R. WESSEL[2];
[1]Washington Univ. In St.Louis, University City, MO; [2]Physics, Washington Univ. In St.Louis, St. Louis, MO

Abstract: Cortical neurons *in vivo* fire irregularly, the distribution of time-averaged firing rates resembles a lognormal function, and the collective activity is coordinated, often showing signs of scale-free dynamics. These non-trivial properties of cortical activity pose the question as to what clues they provide about the underlying cortical microcircuits. Previous theoretical attempts to address this question have focused either on the single-neuron spiking or on the population activity, but not on both. Here we ask to what extent the statistical properties of single-neuron and network activity can arise together as emergent properties of a structured recurrent network. We use a network of identical binary probabilistic integrate and fire model neurons. Connections are excitatory and are chosen at random with a sparse connectivity, thus resulting in a distribution of the number of inputs (in degree) for all neurons. All chosen connections have

identical synaptic strength and synaptic time constant. Varying the synaptic strength from low to high, the network undergoes a transition from a phase of short-lasting population activity (sub-critical), to a highly variable (scale-free) population activity (critical) phase, followed by a phase of long-lasting population activity (super-critical). Surprisingly, right near the critical point of network activity, the single neuron spiking undergoes a phase transition from Poisson spike trains to irregular spiking, characterized by a broad distribution of the coefficient of variation (CV) with a population average above 1. However, using a model of synaptic depression we showed that adding adaptation to the network shifts this transition from the critical point toward the super-critical regime. Moreover, It is shown that further increasing the average synaptic length will eventually make the network to end up at a third phase of regular spiking. In the irregular spiking regime, the distribution of CVs in a network of identical neurons results from the distribution of in degrees; the CV increases with increasing in degree. The distributions also turn out to remain very broad even over very long simulation runs. In addition, imposing a modular connectivity to the network is capable of producing lognormally distributed rates. In conclusion, this investigation indicates that irregular spiking and scale-free network activity can coexist as emergent properties of a recurrent network with structure, without any need to impose certain characteristics to the dynamics of individual neurons.

Disclosures: **Y. Karimipanah:** None. **Z. Ma:** None. **R. Wessel:** None.

Poster

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Title: Dynamics of cortical noise correlations during vision

Authors: ***N. WRIGHT**[1], M. HOSEINI[1], W. P. CLAWSON[2], J. POBST[1], T. CROCKETT[1], W. L. SHEW[3], R. WESSEL[1];

[1]Physics, Washington Univ. In St. Louis, Saint Louis, MO; [2]Electrical Engin., [3]Physics, Univ. of Arkansas, Fayetteville, AR

Abstract: The cortex is an interaction-dominated network; the majority of inputs to a neuron have their source in other cortical neurons. Spontaneous and evoked events dynamically reorganize these connections via adaptation. Not surprisingly, then, neuronal sensory responses can be highly variable across repetitions of a given stimulus, and this variability can be coordinated across a population of neurons. This "noise correlation" likely affects population coding, and given its source, lies at the heart of the interaction between sensory input and cortical circuitry in sensory representation. Understanding this interaction requires a reliable measure of noise correlations in ongoing and evoked cortical activity. This fundamental issue has yet to be fully addressed. To this end, we have simultaneously recorded the membrane potential (V) from up to two cortical neurons, as well as the nearby local field potential (LFP), in the turtle eye-attached wholebrain *ex vivo* preparation. In a parallel set of experiments, we monitored the LFP at several locations using a microelectrode array. Each LFP reflected the net activity in a small volume of cortex, and each membrane potential reflected the spiking activity of the pool of presynaptic neurons. We calculated noise correlations (the Pearson cross-correlation functions of the band-pass filtered recordings (0.1 to 100 Hz) after trial averages had been subtracted) for V-V, V-LFP, and LFP-LFP pairs before and after visual stimulation. We find that for V-V and V-LFP pairs, noise correlation values, and the degree to which they are modulated by visual stimulation, vary greatly across trials and pairs, leading to across-trial and across-pair averages that are small relative to some single-trial values. Nevertheless, there can be significant population trends, depending on the frequency band considered. In contrast, LFP-LFP pairs were remarkably consistent across trials and pairs. We reproduced several aspects of these results using a simple network model. We repeated this analysis for spontaneous oscillations, that is, large V and LFP events that occurred in the absence of visual stimulation. We find a frequency-dependent relationship between noise correlations for the two oscillation types. We interpret this result in the context of the cortical architecture, and hypotheses relating the frequency content of inputs to the location of their sources. Together, these results contribute to a clearer picture of the nature of noise correlations in the visual cortex, how they can depend on recording technique, and how sensory input engages the inherent intracortical connectivity to generate the visual response.

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Poster

059. Population Coding in Striate Cortex

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Support: RVR: HHMI Predoctoral Fellowship

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NIH Grant NS090473

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Title: Dissecting the inhibitory mechanisms of reliable coding in mouse primary visual cortex

Authors: *R. V. RIKHYE, M. SUR;
Dept of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Neurons in the primary visual cortex (V1) respond to full-field natural scenes with spike trains that are highly reliable between trials. While it has been argued that local inhibitory interneurons are responsible for modulating reliable coding, no study has yet systematically detailed the role of different interneuron subtypes. Our goal was to show how Parvalbumin (PV), Somatostatin (SST) and Vasoactive Intestinal Peptide (VIP) expressing interneurons modulate reliable coding in mouse V1. Specifically, we aimed to: (1) show how subnetworks of these interneurons process natural scenes and (2) determine how they contribute to reliable coding. To address these questions, we performed *in vivo* two-photon calcium imaging in awake, head-fixed mice by conditionally expressing GCaMP6f in PV, SST or VIP neurons. This allowed us to minimize the effect of contamination from nearby excitatory neurons and permitted us to study population coding within these interneuron subnetworks. SST neurons also preferred lower spatial frequencies than PV neurons, consistent with their role in integrating information from a larger visual area. Not surprisingly, VIP neurons responded poorly to gratings. PV neurons responded strongly, but unreliably, to full-field natural scenes. In contrast, SST neurons were more selective and were highly reliable between trials. SST cell reliability was comparable to excitatory neurons. This suggests that SST neurons are selectively driven by specific features in natural scenes and, consequently, provide reliable dendritic inhibition on their target cells. We also found that VIP neurons responded more strongly to natural scenes than gratings, suggesting that these interneurons are driven more by “salient” stimuli. Next, we investigated how these interneurons modulated pyramidal cell reliability. To do so, we conditionally expressed ChR2 in both PV or SST neurons and GCaMP6f in pyramidal neurons. We reasoned that reliability arose due precisely timed excitatory (E) and inhibitory (I) synaptic currents. Thus, we used a stimulation protocol to decorrelate these E- and I-currents in pyramidal cells. Specifically, we pulsed a blue LED for 100ms at random times during a natural movie. This allowed us to activate PV/SST neurons during periods when pyramidal cells were most reliable. We

discovered that activating SST neurons during epochs of reliability increased reliability. In contrast, stimulating PV neurons reduced reliability. Taken together, our work demonstrates that SST neurons play an important role in shaping the reliability of pyramidal cell responses to natural scenes in mouse visual cortex.

Disclosures: R.V. Rikhye: None. **M. Sur:** None.

Poster

059. Population Coding in Striate Cortex

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CMU ProSEED/BrainHub

Title: Scaling properties of dimensionality reduction for neural populations and network models

Authors: *R. WILLIAMSON[1,2], B. COWLEY[1], A. LITWIN-KUMAR[3], B. DOIRON[2], A. KOHN[4], M. SMITH[2], B. YU[1];
[1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]Columbia Univ., New York City, NY; [4]Albert Einstein Col. of Med., Bronx, NY

Abstract: Recent studies have applied dimensionality reduction to neural population activity to uncover evidence of neural mechanisms underlying decision-making, motor control, olfaction, working memory, and other behavioral tasks. These techniques provide quantitative measures of population-level structure in neural data. However, it is currently unclear how the outputs of dimensionality reduction (e.g., dimensionality and percentage of overall variance that is shared among neurons) are influenced by the number of recorded neurons and trials, as well as the underlying network structure. To this end, we applied factor analysis to recordings of spontaneous activity from Utah arrays implanted in visual area V1 of anesthetized macaque monkeys. Factor analysis can separate the raw spike count covariance matrix into a shared

component and a private component. We calculated the dimensionality of the population activity and the percentage of spike count variance shared with other recorded neurons (termed 'percent shared'). We repeated this analysis for various neuron and trial counts for each data set. We observed that dimensionality increased with neuron count and trial count, while percent shared remained stable over a wide range of neuron and trial counts. To extrapolate our findings to a larger number of neurons and trials, we investigated whether network models with a balance of excitation and inhibition have similar scaling properties as biological networks. We simulated neurons from networks with two difference structures, non-clustered (i.e., random connections) and clustered (i.e., structured connections), and applied the same analysis used on experimental data. We found that clustered network models showed similar scaling trends as V1 recordings within the experimental range of neuron and trial counts. Non-clustered networks did not show these trends. We then scaled up the number of neurons and trials for the network models and found that the clustered network dimensionality plateaued and percent shared remained stable with increasing neuron and trial count. Lastly, we varied the number of clusters represented in the set of neurons sampled. We observed that dimensionality increased with cluster representation, while percent shared remained stable. Taken together, our results show that the outputs of dimensionality reduction depend on the number of trials and neurons sampled, the underlying network structure, and the number of clusters represented in the neurons sampled. More broadly, these results demonstrate how spiking network models can provide vital context to population analyses based on a limited sampling of neurons and trials in experiments.

Disclosures: **R. Williamson:** None. **B. Cowley:** None. **A. Litwin-Kumar:** None. **B. Doiron:** None. **A. Kohn:** None. **M. Smith:** None. **B. Yu:** None.

Poster

059. Population Coding in Striate Cortex

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Title: Adaptive tuning of mutual information in visual cortex

Authors: *W. CLAWSON[1], N. C. WRIGHT[2], J. YANG[1], R. WESSEL[2], W. L. SHEW[1]; [1]Univ. of Arkansas, Fayetteville, AR; [2]Washington Univ., St. Louis, MO

Abstract: Coding mechanisms in visual cortex adapt to accommodate changes in sensory input. It is often supposed that such adaptation serves to improve coding of the visual input. Conversely, it stands to reason that during a transient period following a large change in visual input, coding will be relatively less effective. Such adaptive changes in coding have largely been studied at the level of one or two neurons. However, sensory information processing in the cortex is not carried out by isolated neurons; it depends on dynamic interactions among large populations of neurons . How such population dynamics emerge from many interacting single neurons is a highly non-trivial problem and remains poorly understood. Our recent experiments in visual cortex of turtles suggest a new principle relating adaptation and population dynamics. We found that adaptation tunes the interactions among cortical neurons to a special regime of population dynamics, called criticality. The transient response following a large change in stimulus was not consistent with criticality. The defining feature of criticality is that it is poised at the boundary between two distinct regimes of population dynamics: one with strongly coordinated population activity, the other with weak coordination. The possibility that adaptation tunes the cortex to criticality is important because other studies suggest that information transmission from stimulus to population response is optimized in networks which operate at criticality. This possibility is in line with the idea that adaptation improves coding, but has not yet been tested directly in any experiment. This is the aim of the work we present here. We studied the *ex vivo*, eye-attached, whole-brain preparation in turtles. We presented dynamic visual stimulation to the retina and measure population activity with high-density, three-dimensional microelectrode arrays. We found that foreground stimuli were better distinguished by population neural response during times when the system is more adapted (i.e. closer to steady state). Distinguishability of stimuli was quantified by computing mutual information between stimulus and response. Compared to transient visual response, steady-state visual response had higher mutual information and showed signatures of being closer to criticality. These findings support the hypothesis that adaptation in visual cortex tunes population dynamics to criticality, a regime that is favorable for discriminating visual stimuli.

Disclosures: W. Clawson: None. **N.C. Wright:** None. **J. Yang:** None. **R. Wessel:** None. **W.L. Shew:** None.

Poster

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Title: Intermittent ensemble oscillations emerge dynamically from cortical circuits of irregular spiking neurons

Authors: *M. HOSEINI[1], J. POBST[1], N. WRIGHT[1], W. CLAWSON[2], W. SHEW[3], R. WESSEL[1];
[1]Physics, Washington Univ. In St. Louis, Saint Louis, MO; [2]Electrical engineering, [3]Physics, Univ. of Arkansas, Fayetteville, AR

Abstract: The ubiquity of local field potential (LFP) oscillations in cerebral cortex begs the question as to what these mesoscopic phenomena reveal about the underlying microcircuits. To address this question, we recorded neuronal oscillations in the visual cortex of turtle. This preparation was chosen for two reasons. First, the LFP oscillations generated in this cortex are sufficiently large to permit the single-trial analysis of neuronal oscillations without the need for averaging. Second, the turtle *ex vivo* eye-attached whole-brain preparation facilitates the combined LFP and simultaneous membrane potential recording from adjacent neurons. The single-trial analysis revealed six important features of cortical oscillations. (i) Evoked and ongoing epochs of LFP oscillations are qualitatively similar. Both exhibit large increases in power with peaks in multiple frequency bands. (ii) Epochs of LFP oscillations are intermittent and of variable (trial-to-trial) peak frequency and duration. (iii) Single neuron spiking remains largely irregular and of low rate (compared to oscillation peak frequency) during epochs of LFP oscillations. (iv) Throughout an LFP oscillation epoch, the phase drifts, thus indicating the absence of autocoherence. (v) The fluctuating aspects of LFP oscillations can co-vary across spatially separate cortical recording sites. However, the grouping of recording sites by coherence can fluctuate from trial-to-trial. (vi) Membrane potential oscillations exhibit a large increase in power, but are broadband in the 0 to 100 Hz range, both for ongoing and evoked epochs of oscillation. We reproduced the observed intermittent ensemble oscillations in a model network, consisting of excitatory and inhibitory model neurons with the characteristics of regular-spiking pyramidal neurons, and fast-spiking and low-threshold spiking interneurons. In conclusion, this work suggests biologically plausible mechanisms for the stochastic generation of episodes of intermittent ensemble oscillations with irregular spiking neurons in cortical circuits.

Disclosures: **M. Hoseini:** None. **J. Pobst:** None. **N. Wright:** None. **W. Clawson:** None. **W. Shew:** None. **R. Wessel:** None.

Poster

059. Population Coding in Striate Cortex

Location: Hall A

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Program#/Poster#: 59.11/J46

Topic: D.04. Vision

Title: Sparseness and redundancy of visual stimulus encoding in primary visual cortex

Authors: *S. P. DUARTE, J. SCHUMMERS;
Max Planck Florida Inst., Jupiter, FL

Abstract: Neurons in primary visual cortex (V1) respond preferentially to stimulus features within a particular range, such as an oriented bar within a limited range of orientations. From these responses, we can generate tuning curves and then extract the neuron's preferred orientation. By plotting the preferred orientations of populations of neurons across cortical space, we can construct an orientation map. Orientation maps have been well described both at the large scale and cellular levels, where previous studies have shown that orientation transitions smoothly around a central pinwheel and nearby neurons have similar preferred orientations. However, the responses of V1 neurons have been probed using other stimulus features, including direction of motion, location in visual space, spatial and temporal frequency, and preferred eye. The cellular level organization of these other functional maps have been less well described. Additionally, how these functional maps are superimposed on the same cortical space is not well understood. For example, does a single neuron have tuned responses to all of the stimulus features, suggesting that a single neuron may encode all features of a unique stimulus? Or is a single neuron tuned to only one or two features, suggesting that a unique stimulus must be encoded by a population of neurons. Neither the sparseness nor the redundancy of the neural code in V1 have been directly quantified. Here we use two-photon imaging of genetically encoded calcium indicators (GCaMP6s) in the visual cortex of ferrets to explore these questions. We present a battery of simplified visual stimuli in order to extract the receptive field and tuning properties for a variety of stimulus features for hundreds of neurons over the same area of visual cortex. We then construct multiple large scale functional maps of the same region at cellular resolution, including retinotopy, orientation, direction, spatial and temporal frequency and ocular dominance. From these data, we can then examine the range of stimulus features being encoded by individual neurons, as well as how groups of neurons overlap in their representation.

Ultimately, our goal is to determine the level of sparseness and redundancy used in V1 to encode simple visual stimuli, and then extend these principles to the encoding of natural scenes.

Disclosures: S.P. Duarte: None. **J. Schummers:** None.

Poster

059. Population Coding in Striate Cortex

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Support: BFNT 01GQ0840 (MK)

F32EY022001 (GBS)

RO1EY011488 (DF)

Title: Early cortical spontaneous activity reflects the structure of mature sensory representations

Authors: *B. HEIN[1], G. B. SMITH[2], D. WHITNEY[2], K. NEUSCHWANDER[1], D. FITZPATRICK[2], M. KASCHUBE[1];
[1]Frankfurt Inst. For Advanced Studies, Frankfurt, Germany; [2]Functional Architecture and Develop. of Cerebral Cortex, Max-Planck Florida Inst., Jupiter, FL

Abstract: Although spontaneous patterns of neural activity are thought to play an important role in the development of cortical circuits, relatively little is known about the structure of spontaneous activity in the developing cortex and its relation to mature sensory representations. We sought to determine how early patterns of spontaneous activity are related to stimulus evoked patterns in the same animal later in development. Here, we took advantage of the columnar architecture in ferret visual cortex to visualize patchy patterns of spontaneous activity prior to the onset of visual experience and the emergence of the orientation preference map. We used the highly sensitive calcium indicator GCaMP6s to reveal population activity on a single trial basis in chronic recordings of the developing ferret visual cortex. Novel analytical approaches were used to uncover interpretable statistical relations from these data. Prior to eye opening, the correlation structure of spontaneous cortical activity displays robust columnar patterns that resemble the mature organization of the orientation preference map. Although visual stimulation through the closed eyelids evokes strong patterns of activity prior to eye opening, the orientation preference map can only be evoked by visual stimulation after eye opening. We conclude that early spontaneous patterns of cortical activity exhibit an orderly

columnar structure that forms the basis for building sensory evoked representations during cortical development.

Disclosures: **B. Hein:** None. **G.B. Smith:** None. **D. Whitney:** None. **K. Neuschwander:** None. **D. Fitzpatrick:** None. **M. Kaschube:** None.

Poster

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Title: Sibling rivalry and cooperation among excitatory neurons in the neocortex

Authors: *C. R. CADWELL[1], X. JIANG[1], P. BERENS[1,2,3,4], P. G. FAHEY[1], D. YATSENKO[1], E. FROUDARAKIS[1], A. S. ECKER[1,2,4,5], A. S. TOLIAS[1,6];
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Bernstein Ctr. for Computat. Neurosci., Tübingen, Germany; [3]Inst. for Ophthalmic Res., [4]Werner Reichardt Ctr. for Integrative Neurosci. and Inst. of Theoretical Physics, Univ. of Tübingen, Tübingen, Germany; [5]Max Planck Inst. for Biol. Cybernetics, Tübingen, Germany; [6]Dept. of Computat. and Applied Mathematics, Rice Univ., Houston, TX

Abstract: The mammalian neocortex carries out complex mental processes such as cognition, perception and decision-making through the interactions of billions of neurons connected by trillions of synapses. We are just beginning to understand how networks of neurons become wired together during development to give rise to cortical computations. Recent studies have

shown that excitatory cortical neurons with a shared ontogenetic lineage form vertical columns spanning multiple cortical layers and that these "sister cells" are more likely to be synaptically connected to each other than to nearby, unrelated neurons. However, the precise wiring diagram between sister cells is unknown. Here we show that connectivity between sister cells depends on the laminar position of the pre- and post-synaptic neurons. In contrast to previous studies, we find that although sister cells residing in different cortical layers are more likely to be connected, sister cells located within the same layer are less likely to be connected to each other compared to distance-matched controls. Avoidance of cells that receive common input may be a fundamental principle of information processing within a cortical column. Our findings challenge the prevailing hypothesis that shared developmental lineage is always associated with an increase in connectivity, and suggest that both attraction and repulsion play an important role in shaping cortical circuits.

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Poster

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Topic: D.04. Vision

Support: NIH Grant 2R01EY011488-17

Title: Representation of the polarity of luminance transitions in layer 2/3 neurons of ferret visual cortex

Authors: *D. E. WHITNEY[1], G. B. SMITH[1], M. KASCHUBE[2], D. FITZPATRICK[1]; [1]Neurosci., Max Planck Inst., Jupiter, FL; [2]Frankfurt Inst. for Advanced Studies, Frankfurt am Main, Germany

Abstract: We recently reported the existence of an ON - OFF map in the superficial layers of ferret visual cortex. Increments or decrements in uniform luminance were found to drive transient responses in largely complementary sets of layer 2/3 neurons. Many layer 2/3 neurons respond preferentially to the presentation of either uniform luminance transitions or grating stimuli; others respond well to both stimuli. Neighboring neurons in layer 2/3 tend to exhibit similar response ratios for uniform luminance transitions versus grating stimuli. To further explore the relationship between the representation of luminance and orientation in layer 2/3, we

examined cortical responses to a drifting edge stimulus, containing a single-oriented contour defined by an ON or OFF luminance transition. Using *in vivo* wide-field epifluorescence calcium imaging, we find that population responses to this stimulus reflect both the polarity of the luminance transition and the contour orientation. The majority of these responses are best described as an intersection of the ON - OFF and orientation maps. Two-photon calcium imaging of layer 2/3 neurons further reveals that ON - OFF discriminability improves in almost all neurons at their preferred orientation. Likewise orientation selectivity is greater in almost all neurons when luminance transitions at the edge boundary match their preferred polarity. Taken together, these results emphasize that sensitivity to the polarity of luminance transitions is a fundamental property of layer 2/3 neurons. Consistent with this idea, we find that ON - OFF and orientation maps exhibit nearly complete coverage across the visual cortex, with complete representations of ON and OFF throughout orientation space. Notably, this coverage appears to occur despite the absence of orthogonality between the two maps.

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Poster

059. Population Coding in Striate Cortex

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Topic: D.04. Vision

Support: KAKENHI 26640019

Title: Experience-dependent and independent development of feature-selective synchronization in rat visual cortex

Authors: ***A. W. ISHIKAWA**, Y. KOMATSU, Y. YOSHIMURA;
Natl. Inst. For Physiological Sciencesphysi, Okazaki, Japan

Abstract: Synchronous firing among visual cortical neurons is considered to play important roles in visual information processing. We investigated the development of synchronized firing between adjacent neurons in primary visual cortex using immature rats at postnatal 13-15 days (P13-15) just after eye opening and three groups of P24-28 rats raised in a normal visual environment without (normal rearing) or with lid suture of both eyes from the day of eye opening (binocular deprivation), or in darkness from birth (dark rearing). We conducted single-unit recordings from multiple neurons located across all cortical layers using a multichannel electrode

and quantified the synchrony of visually-evoked spikes simultaneously recorded from pairs of neurons by computing the cross-correlogram of their spike trains. In normally reared rats at P24-28, synchronized firing preferentially occurred in neuron pairs located in the upper layer (layer 2-4) when the neuron pairs shared similar visual responsiveness. This feature-selective synchronization in the upper layer was almost absent or weak in immature rats and rats subjected to either dark rearing or binocular deprivation. In normally reared rats at P24-28, the dependence of synchronization on visual response similarity in neuron pairs located in the lower layer (layer 5-6) was weaker than that found in the upper layer. A similar weak dependence in the lower layer was also found in rats just after eye opening and visually deprived rats at P24-28. These results showed that feature-selective synchronized firing in the upper layer was established only when rats were reared under a normal visual environment after eye opening. On the other hand, our results showed that weak feature-selective synchronization was already present in the lower layer just after eye opening and the synchronization remained almost unchanged thereafter, independent of visual experience. Therefore, the development of synchronous firing in the primary visual cortex seems to be mediated by different mechanisms in the upper layer, which provides output mainly to higher cortical areas, compared with the lower layer, which provides output mainly to subcortical regions.

Disclosures: **A.W. Ishikawa:** None. **Y. Komatsu:** None. **Y. Yoshimura:** None.

Poster

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Support: NIH F32EY022001 (GBS)

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Title: Patterns of neural activity in visual cortex of the developing ferret: a comparison of awake and anesthetized states

Authors: ***G. B. SMITH**[1], D. E. WHITNEY[1], B. HEIN[2], K. NEUSCHWANDER[2], M. KASCHUBE[2], D. FITZPATRICK[1];

[1]Max Planck Florida Inst. For Neurosci., Jupiter, FL; [2]Frankfurt Inst. for Advanced Studies, Frankfurt am Main, Germany

Abstract: During development, spontaneous activity is thought to play a key role in patterning the central nervous system prior to the onset of sensory experience, supplementing molecular cues and bridging the gap to experience-dependent mechanisms. In parallel work, we show that the spatial structure of spontaneous activity in the developing visual cortex prior to eye opening is highly correlated with the future orientation map, at a time when the orientation map cannot be evoked through visual stimulation. A key caveat in these experiments, and in several other studies exploring the role of spontaneous activity, is that they were conducted in anesthetized animals. The effects of anesthesia on both stimulus evoked and spontaneous activity remain unclear, with some studies reporting dramatic changes under anesthesia. Here we report the development of a head-restrained preparation for longitudinal awake imaging in the developing ferret, utilizing both wide-field epifluorescence and two-photon imaging of GCaMP signals. We sought to explicitly test the effects of anesthesia on spontaneous and evoked activity in the ferret prior to and shortly following eye opening. Patterns of spontaneous and evoked activity exhibited a prominent modular structure in both the anesthetized and awake states. Under anesthesia, spontaneous activity takes the form of discrete events interleaved with periods of reduced activity. In contrast in the awake cortex, the frequency of events is markedly increased, and events often occur in rapid succession with infrequent quiescent periods. Notably, despite the striking differences in temporal dynamics, the modular patterns of spontaneous and evoked activity are highly similar between awake and anesthetized states, with similar regions tending to be coactive. Similar to results in anesthetized animals, spatial patterns of spontaneous activity recorded prior to eye opening strongly resemble the future orientation map that emerges day later. Therefore, although anesthesia dramatically affects the temporal structure of activity, it does not alter its underlying structure.

Disclosures: G.B. Smith: None. **D.E. Whitney:** None. **B. Hein:** None. **K. Neuschwander:** None. **M. Kaschube:** None. **D. Fitzpatrick:** None.

Poster

059. Population Coding in Striate Cortex

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Topic: D.04. Vision

Support: Simons Collaborations on the Global Brain

Title: The structure of response correlation matrix is stable in the primate visual cortex and reveals the anatomical boundary of V1 and V2

Authors: S. ESTEKI, L. E. HALLUM, V. GARCÍA-MARÍN, J. G. KELLY, *R. KIANI;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Correlations between neural responses have profound effects on the ability of neural populations to compute and encode information. However, the organization of these correlations and the mechanisms that give rise to them are not well understood. Here we investigated the pattern of correlations in the primary and secondary visual cortices (V1 and V2). We used 96-channel microelectrode arrays to simultaneously record visually-evoked and spontaneous activity of tens of neurons in the vicinity of V1-V2 border in two anesthetized macaque monkeys. Histology following the experiments revealed that nearly half of the electrodes were located in each area and recordings were largely limited to layer IV and bottom of layer III. We calculated spike count correlations of all pairs of recorded units, forming a correlation matrix. We found that, although the magnitude of the correlations may depend on the stimulus, the overall structure of the correlation matrix is largely stable; the ranking of pairwise correlations is preserved across all conditions and epochs, including the spontaneous activity. Furthermore, the correlation matrix structure reflects the V1-V2 border -- neurons within each area are more positively correlated with each other than across areas. In fact, unsupervised clustering algorithms could retrieve the location of the anatomical boundary even in the absence of a priori information about the electrode locations. We show that this cortical parcellation is not shaped solely by the distance separating units, their cortical layer, overlap of RFs, orientation preference, or ocular dominance. Rather, the correlation matrix structure is shaped mainly by "common noise": spontaneous activity or the residual fluctuations around the stimulus-evoked response. These fluctuations are temporally broadband across several orders of magnitude (0.01-20Hz) and could not have been caused by low frequency fluctuations in cortical excitability. We suggest that the population response dynamics which give rise to the stability of the correlation structure are furnished by intrinsic connectivity within an area, V1 or V2. The anatomical pattern of lateral connections -- dense connectivity within areas and minimal crossing across areal boundary (Rockland & Lund 1982, 1983) -- supports our hypothesis. The stability of the structure of correlation matrix in visual cortices is reminiscent of previous reports on the primate prefrontal cortex (Kiani et al, Neuron 2015), indicating an organizational principle preserved across sensory and association cortices.

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Poster

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Alfred P. Sloan Award

Title: Behavioral-state dependence of cortical activity and visual encoding in a genetic model of autism

Authors: ***M. VINCK**, J. MOSSNER, J. CARDIN;
Yale Univ., New Haven, CT

Abstract: Dysregulation of GABAergic inhibitory function in the brain is a candidate mechanism underlying autism-related neurodevelopmental disorders, but little is known about the relationship between GABAergic cellular dysfunction, altered neural network activity, and cognitive impairments in autism. Global genetic loss of MeCP2 in mouse models recapitulates many Rett phenotypes, including impaired social interactions, cognitive deficits, and repetitive movements, but the cell type-specific role of MeCP2 signaling and the contributions of specific interneuron populations to these phenotypes remain unclear. Recent studies have highlighted the dependence of cortical activity and sensory encoding on behavioral state. Here, we study the behavioral-state dependence of cortical activity and visual encoding while deleting MeCP2 from specific GABAergic interneuron populations (SOM, PV, VIP), using a combination of single-unit and local field potential (LFP) recordings. We find altered cortical activity patterns around behavioral state transitions, revealing a role of the MeCP2 gene in regulating the way in which bottom-up sensory activity is integrated with spontaneous, internal activity.

Disclosures: **M. Vinck:** None. **J. Mossner:** None. **J. Cardin:** None.

Poster

059. Population Coding in Striate Cortex

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Topic: D.04. Vision

Title: Method for analyzing neuronal response variability based on frequency response amplitude

Authors: *A. RAHIMABADI[1], M. ALIKHANI[1], V. DAVOODNIA[1], M. ZANGANE[1], H. RAHIMI NASRABADI[1], R. LASHGARI[1,2,3];
[1]Sch. of Cognitive Sci., Inst. For Res. In Fundamental Sci., Tehran, Iran, Islamic Republic of; [2]school of electrical engineering, iran university of science and technology, Tehran, Iran, Islamic Republic of; [3]national brain mapping center, Shahid Beheshti medical university, Tehran, Iran, Islamic Republic of

Abstract: In primary visual cortex, the variance of neuron's firing rate is directly proportional to its mean firing rate (Tolhurst et al., 1983) and the firing rate is generally higher in complex cells than simple cells (Schiller et al., 1976). Here, we introduce a new method for measuring response variability based on the mean variance of the frequency response amplitude (MVFRA). We measured the relation between response variability in the time domain and the frequency domain and quantified the mean variance of the frequency response amplitude in simple cells and complex cells. To measure the response variability of neurons, we used chronically implanted ultra-thin multi-electrode array with impedances of 1-3 MΩ (Swadlow et al, 2005; Lashgari et al, 2012) in primary visual cortex (V1 area) and measured the neuronal responses to gratings drifting at 2 Hz for 2-3 seconds. The gratings could have different orientations (8 orientations with 16 directions) or luminance contrasts (8 contrast). Preliminary results indicate that the stimulus that generate the maximum firing rate not always generate the lowest response variability and that responses with the highest firing rate can be just as variable as those with lower firing rate. Our results suggest that the preferred stimulus of a V1 neuron is better described by the lowest response variability than by the maximum firing rate. These results may have implications to understand how stimuli are best represented by neuronal responses and suggest that responses with low variability may be more reliable for higher brain functions such as attention and decision making.

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Poster

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Kavli Institute for Brain Science, Columbia University

Gatsby Initiative in Brain Circuitry, Columbia University

Title: The stabilized supra linear network (SSN) model explains feature-specific surround suppression in V1

Authors: D. OBEID[1], *K. D. MILLER[2];
[1]Ctr. for Theoretical Neurosci., [2]Cntr Theoretical Neurosci, Columbia Univ., New York, NY

Abstract: We have recently presented the SSN model of sensory cortical integration (Rubin et al., Neuron 2015; Ahmadian et al., Neural Comp. 2013). The main ideas are (1) Individual neurons have supralinear (power-law) input/output (I/O) functions, so that (2) the gain of neural responses (change in response per change in input; slope of the I/O function) increases with network activation; (3) for weak activation (low gain), neurons are only weakly coupled, dominated by their external input (from outside the local network), and responses to different inputs sum supralinearly following the supralinear I/O function; (3) increasing network activation (increasing gain) yields potential instability _ fluctuations in excitatory (E) cells would drive even bigger E cell responses via E->E connections; (4) in broad parameter regimes, the network instead dynamically stabilizes through feedback inhibition; (5) this stabilization is achieved by recurrent input largely cancelling feedforward input, so that net input grows much more slowly than linearly with the feedforward input. As a result, the network adds responses to different inputs sublinearly. The SSN with plausible models of cortical circuitry explained multiple aspects of cortical behavior, including “normalization” (sublinear response summation) that becomes more linear or supralinear for weak inputs, and in which both E and inhibitory (I) neurons are normalized; and orientation-tuned surround suppression in which (i) a strong surround becomes more weakly suppressive or facilitating for a weak center input; (ii) summation field sizes shrink with contrast; (iii) both E and I neurons surround suppress, so the net inhibition received by cells is decreased by surround suppression (Ozeki et al., Neuron 2009; Pecka et al., Neuron 2014). Here we show the SSN model replicates two other sets of results: (1) surround modulation is maximal when the orientation of the surround stimulus matches that of the center stimulus, even when the center stimulus is non-optimal for the cell (Shushruth et al, J. Neurosci. 2012); (2) For surround suppression of plaid center stimuli, a surround with orientation matching one component of the plaid more strongly suppresses that orientation’s contribution to the neural response, rather than non-specifically suppressing responses (Trott et al, J. Neurosci. 2015). These, and the decrease in inhibition with surround suppression in a large-scale model (D. Obeid & K.D. Miller, Cosyne 2015), required the network to have stronger local connectivity

than our previous studies, but previous results are preserved. We are now studying transient dynamics and signal propagation in the network.

Disclosures: D. Obeid: None. **K.D. Miller:** None.

Poster

059. Population Coding in Striate Cortex

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 59.21/K8

Topic: D.04. Vision

Support: The budget of Kyoto Sangyo University

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CiNet Preproject

MEXT Project "Creating Hybrid Organs of the Future"

Title: Vertical and horizontal structures of neuronal correlations in the cat primary visual cortex

Authors: *H. TANAKA[1], H. TAMURA[2], I. OHZAWA[2];
[1]Kyoto Sangyo Univ., Kyoto-shi/Kyoto, Japan; [2]Osaka Univ., Suita, Japan

Abstract: The firing patterns of nearby cortical neurons are often temporally correlated, which may contribute to numerous aspects of cortical processing. It is known that the correlation occurs on a variety of timescales. In particular, many previous studies noted precise correlation on a timescale of milliseconds and loose correlation on a timescale of tens of milliseconds. In order to fully characterize cortical organization of these correlations, we investigated their spatial extent, laminar organization, and dependence on receptive field (RF) similarities. By penetrating four-shaft arrays of microelectrode (a4x8x200_400_177, NeuroNexus) perpendicularly into anesthetized cat area 17 and 18 (between A3 and P8 and between L1 and L3), we simultaneously recorded neuronal activities across layers within a horizontal distance of 1.2 mm in response to flashed sequences of sinusoidal gratings or drifting gratings. We computed cross-correlograms (CCGs) based on these activities for neuronal pairs (n= 2426) separated by various distances in horizontal or vertical directions. In order to measure the strength of precise and loose correlations separately, we, using a median filer, dissociated the CCGs into narrow peak

components with a width of <= 8 ms and broad peak components with a width of > 8 ms. We found that loose correlation was widely observed for neuronal pairs horizontally and vertically separated over the whole distance range regardless of the layers. But the incidence was substantially lower in layer 4 than in other layers particularly for short horizontal distances (20% for layer 4 vs. 40~80% for other layers). Loose correlation also accompanied a consistent delay in firing that was monotonically related to the vertical, but not horizontal, distance between the paired neurons (5.85 ms/mm and 7.3 ms between L6 and L2/3). These patterns of loose correlation are consistent with the previous proposal that this correlation is mainly mediated by feedback projections from the higher cortical areas. On the other hand, precise correlation was horizontally much more limited with the incidence dropping sharply within 0.4 mm for all layers. Vertically, this correlation was typically observed for pairs of neurons in the same layers, without substantial difference of correlation strength between layers. Furthermore, precise correlation was predominantly seen for pairs with similar RF properties, whereas loose correlation was seen even in pairs showing dissimilar properties. Our results show that neuronal correlations in V1 show markedly different structures for horizontal and vertical dimensions depending on correlation time-scales.

Disclosures: **H. Tanaka:** None. **H. Tamura:** None. **I. Ohzawa:** None.

Poster

059. Population Coding in Striate Cortex

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Title: Adaptation to sensory input tunes visual cortex to criticality

Authors: ***W. L. SHEW**[1], W. CLAWSON[1], Y. KARIMIPANAH[2], J. POBST[2], N. C. WRIGHT[2], R. WESSEL[2];
[1]Univ. of Arkansas, Fayetteville, AR; [2]Washington Univ., St. Louis, MO

Abstract: A long-standing hypothesis at the interface of physics and neuroscience is that neural networks self-organize to the critical point of a phase transition, thereby optimizing aspects of

sensory information processing. This idea is partially supported by strong experimental evidence for critical dynamics observed in the cerebral cortex, but the impact of sensory input on these dynamics is largely unknown. Thus, the foundations of this hypothesis - the self-organization process and how it manifests during strong sensory input - remain unstudied experimentally. Thus, a basic question remains open: does strong sensory input drive cortical network dynamics away from criticality? Indeed, sufficiently strong input may increase the overall excitability of a network by bringing neurons closer to their firing thresholds and potentially tipping the network into a high firing rate regime that is inconsistent with critical dynamics. Previous theoretical work suggests that adaptation, i.e. activity-dependent changes in neural interactions, may serve to mitigate this tendency and maintain criticality. Here we address this problem experimentally in the *ex vivo*, eye-attached, whole-brain preparation in turtles. We presented diverse visual stimuli to the retina and measure population activity in visual cortex using high-density microelectrode arrays. We show, in our experiments and in a computational model, that strong sensory input initially elicits cortical network dynamics that are not critical, but adaptive changes in the network rapidly tune the system to criticality. This conclusion is based on observations of multifaceted scaling laws, predicted to occur at criticality. Our findings establish sensory adaptation as a self-organizing mechanism which maintains criticality in visual cortex during sensory information processing.

Disclosures: **W.L. Shew:** None. **W. Clawson:** None. **Y. Karimipanah:** None. **J. Pobst:** None. **N.C. Wright:** None. **R. Wessel:** None.

Poster

060. Visual cognition: Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 60.01/K10

Topic: D.04. Vision

Title: Difference of VEFs with mental arithmetic tasks and verbal fluency task

Authors: *Y. GOTO;
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Abstract: The aim of this study was to investigate the detailed spatiotemporal brain processes of divided attention which related to two types of mental arithmetic tasks (simple and complex Calculation) and the verbal fluency task using visual evoked magnetic fields. Fourteen healthy volunteers (8 women and 6 men, mean age 27.7±6.8 years) participated in this study. The magnetoencephalography (MEG) was acquired using a whole-head 306-channel sensor array

(Vectorview, ELEKTA Neuromag, Helsinki) that comprises102 identical triple-sensor elements. Each sensor element consists of two orthogonal planar-type gradiometers and one magnetometer. In this study, we analyzed MEG data recorded by the 204-channel planar-type gradiometers. A hemifield black-white checkerboard pattern was phase-reversed at a rate of 1 Hz presenting or left visual hemifield with (task +) or without (task -) mental arithmetic tasks and verbal fluency task. The entire stimulating field subtended an angle of 15°; each individual square of the checkerboard subtended 50' of arc measured at the subject's eye. The mean luminance of stimulation was 30 cd/m2 and the contrast was 97 %. The subjects were instructed to fixate on a red dot placed 0.2° of arc laterally from the stimulated hemifield. The VEFs signals were bandpass filtered between 0.1-1000 Hz. Analog data were digitized at a sampling rate of 1000 Hz/channel, and 100 responses of 350 ms epochs in each session were averaged using a computer. The amplitudes and latencies of VEFs components were measured. Lower amplitude distribution of three distinct components (N75m, P100m, N145m) were identified the contralateral to hemifield stimulation in each task to compare without task. In latency changes by left hemifield stimulation, the mental arithmetic tasks were significantly delayed in comparison with task(-) and successive subtraction in P100m. In right hemifield stimulation, no significant changes were observed. Amplitude changes in N75m on left hemifield stimulation were significantly decreased in mental arithmetic tasks. In addition, the distribution of high-gamma band activities was decreased in the right occipital area with left hemifield stimulation by all tasks (+) condition. In contrast, those were increased in the left occipital area with right hemifield stimulation by the all mental tasks. This study identified and documented that statistical the significantly changes in PR-VEFs were observed during mental arithmetic tasks and verbal fluency task. Our results suggest that these changes are the evidence of the inhibition of the visual information processing in the primary visual cortex by the tasks.

Disclosures: Y. Goto: None.

Poster

060. Visual cognition: Decision Making

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Support: NIH R01-EY019041

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McKnight Scholar award

Title: Impact of visual familiarity on neuronal representations in inferior temporal cortex and behavior

Authors: *K. MOHAN, W. J. JOHNSTON, D. J. FREEDMAN;
Dept. of Neurobio., The Univ. of Chicago, Chicago, IL

Abstract: We have a remarkable ability to recognize a huge number of familiar visual stimuli. Furthermore, the relative novelty or familiarity of stimuli has a marked impact on attention and behavior. For example, the presence of a lion in your living room attracts your attention since it is a novel event that is unexpected and out of context. Here, we explore how familiarity impacts the neuronal representation of visual stimuli, and the relationship between neuronal and behavioral effects of familiarity. Previous work revealed that activity of neurons in the inferior temporal cortex (ITC) show changes in both visual responsiveness and stimulus selectivity as a result of long-term familiarity. In this study, we examined the impact of both long-term and short term (within session) familiarity on neuronal activity in ITC. Monkeys were familiarized with an image set and then trained to perform a dimming-detection task with novel and familiar images (100 images) shown ~50 times each. In the task, a series of images are presented for 400 ms each; on each trial, all images in the sequence have an equal probability of dimming (i.e. a subtle decrease in luminance). The monkey is required to release a lever if he detects dimming or hold on, if he does not. We recorded from 70 ITC neurons in one monkey during the task. We find that among stimulus selective neurons (50/70), novel stimuli evoke significantly higher average firing rates than familiar stimuli, consistent with previous reports. We also examined within-session changes in responses to the novel and familiar stimuli. This revealed distinct sub-populations of neurons which showed either increases or decreases in average firing rate within session, as well as changes in their parameters of stimulus selectivity. We also investigated the behavioral impact of stimulus novelty and familiarity by examining the animals' gaze patterns during free viewing of arrays of novel and familiar stimuli. In each trial, the subject was shown two images simultaneously presented at 3° to the left and right of fixation, and was allowed to freely view the images for 5 s. The images systematically varied in their familiarity - novel, intermediately familiar (~1000 viewings), and highly familiar (~10,000 viewings). We observed a systematic novelty bias in viewing times for both the intermediately and highly familiar stimuli compared to novel stimuli in the early (200-1500 ms) but not late (1700-5000 ms) viewing period. We examine and compare the time course of emergence of neuronal and behavioral familiarity signals. Our results will provide insights into the mechanisms underlying experience-dependent changes in ITC activity and their impact on behavior.

Disclosures: K. Mohan: None. **W.J. Johnston:** None. **D.J. Freedman:** None.

Poster

060. Visual cognition: Decision Making

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Topic: D.04. Vision

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Title: Sequential processing of sensory and decision signals in posterior parietal cortex

Authors: *G. IBOS[1], D. J. FREEDMAN[2];
[2]Neurobio., [1]The Univ. of Chicago, Chicago, IL

Abstract: Neurons of the lateral intraparietal area (LIP) encode a wide range of sensory, cognitive and motor factors, and are known to be engaged during visual decision making tasks in which stimuli must be evaluated and used to flexibly guide action. To better understand the mechanisms by which LIP transforms a sensory signal into decision related encoding, we trained two monkeys to perform a delayed conjunction matching (DCM) task in which they had to indicate whether test stimuli matched a previously a presented sample while ignoring task-irrelevant distractors simultaneously shown in the opposite hemifield. Sample, test and distractor stimuli were high contrast random-dot motion patterns that parametrically varied in both their direction (among 8 evenly spaced directions) and color (8 colors from red to yellow). On each trial, one of two sample stimuli, composed of a conjunction of one direction (90° or 270°) and one color (yellow or red), was shown either inside or outside the receptive field (RF) of the neuron being recorded. Monkeys were rewarded for releasing a lever to test stimuli that matched the sample in direction, color and position. We recorded the activity of 74 LIP neurons, and found that 56/74 neurons differentiated between matching and non-matching test stimuli, or showed selectivity for the visual features of test stimuli. We computed two ROC-based selectivity measures: 1) a “sensory ROC” (sROC), comparing responses to each of the two match stimuli; 2) a “decision ROC” (dROC) comparing responses to match and non-matching stimuli. A sensory-decision Index ((sROC-dROC) / (sROC+dROC)) measured the relative strength of sensory encoding compared to the strength of decision encoding. This revealed a continuum of selectivity with some neurons showing pure visual selectivity, some showing pure decision selectivity, and some showing mixed selectivity. Decision related signals were not related to motor preparation, as few LIP neurons encoded information about match stimuli that were located outside LIP neurons’ RFs. In addition, a support-vector machine classifier was able to accurately decode both the visual features and the behavioral decision associated with match stimuli. An examination of the time-course of visual feature and decision related encoding in LIP revealed a shorter latency of visual (~50 ms after stimulus onset) compared to decision related (~120 ms) signals. Together, this suggests that sequential computations take place within

populations of LIP neurons starting with the integration of sensory information followed by a transformation into decision-related encoding.

Disclosures: G. Ibos: None. **D.J. Freedman:** None.

Poster

060. Visual cognition: Decision Making

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Topic: D.04. Vision

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NIH CRCNS MH092729

NSF CAREER Award

Title: Task dependence of visual and mnemonic encoding in parietal and prefrontal cortices

Authors: *A. SARMA[1], X.-J. WANG[2], D. J. FREEDMAN[1];
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Abstract: Prior studies have shown that neuronal activity in the lateral intraparietal (LIP) area and prefrontal cortex (PFC) can reflect the learned category membership of visual stimuli. A recent study from our group examined how category representations in LIP develop during learning, and how neuronal encoding of working memory in LIP and PFC are influenced by task demands. This study suggested that neuronal representations of working memory in LIP are task specific, while PFC plays a more general role in working memory. Here, we aim to further examine the task dependence of neural representations in LIP and PFC. The primary goal of this project is to understand how task demands influence neuronal encoding in LIP and PFC by recording from these areas while monkeys flexibly switch between feature-matching and category-matching tasks. Two monkeys were previously trained to perform a delayed match to sample (DMS) task using 360° of motion directions as sample and test stimuli. Monkeys released a lever to indicate whether a test stimulus and sample stimulus, separated by a memory delay period, showed the same motion direction. Monkeys were then trained on a delayed match to category (DMC) task (using the same stimuli) in which they indicated whether sample and test stimuli were in the same category, defined by an arbitrary category boundary. For this study, the two monkeys previously trained on the DMS and DMC tasks were trained to switch between

DMS and DMC tasks on a trial-by-trial basis. Monkeys were cued by a colored fixation point to release the lever when sample and test stimuli were either identical matches (DMS rule) or category matches (DMC rule). Motion stimuli and task timings were identical between these two types of trials; only the color of the fixation point was different. Furthermore, 50% of trials had an ambiguous task rule cue until halfway through the delay period. These ambiguous cue trials were indicated through the fixation point by a third color. We recorded from neurons in LIP (N=55) and PFC (N=210) while monkeys switched between DMS and DMC tasks. The results of this experiment will indicate whether neuronal representations in LIP and PFC are task dependent on a short time scale (trial-to-trial). Examination of activity in LIP and PFC will reveal the flexibility and timing of encoded information about motion direction, learned categories, task rule, and match decision during various epochs of the task. Ambiguous cue trials will show whether feature information is encoded in working memory in LIP when multiple later decisions need to be made. Simultaneous recordings in PFC and LIP will reveal how activity in these areas is coordinated under differing task demands.

Disclosures: **A. Sarma:** None. **X. Wang:** None. **D.J. Freedman:** None.

Poster

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Topic: D.04. Vision

Support: NIH Grant R01EY19041

Title: Semi-chronic population recordings from parietal and prefrontal networks during a rapid spatial categorization task

Authors: ***N. Y. MASSE**, J. M. HODNEFIELD, D. J. FREEDMAN;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: How we interact with objects in the environment is often based on their relative spatial location, such as deciding whether to reach for a jar on a high shelf, or whether a car is far enough away to safely cross the street. Both the posterior parietal (PPC) and prefrontal (PFC) cortices have been shown to encode spatial information, but how this information is transformed into a categorical decision is poorly understood. Here, we use a novel, rapid decision-making task to study how spatial information is encoded and categorized in the fronto-parietal network. We trained two monkeys to perform a spatial categorization task using a rapid series of flashed

stimuli. The monkeys maintained visual fixation on a central point while a series of 3 to 60 (mean of 14) white, red and green dot flashes (0.2° radius) appeared within a 13° x 26° zone in one hemifield. Flashes occurred once every 160 ms. The monkeys had 400 ms to manually release a touch bar after a red flash appeared in a 4° x 4° target zone whose center was located 6.5° away from the fixation point, or whether a green flash appeared in an adjacent target zone of equal size and eccentricity. White flashes were to be ignored. The two target zones were fixed throughout the training and recording sessions. The monkeys responded selectively to red and green flashes in their respective target zones (88.6 ± 0.5% response rate when flash occurred in correct target zone, 45.2 ± 1.1% response rate when flash occurred in incorrect target zone). We also trained the monkeys to perform a flash mapping task, which was similar to the task above except that the monkeys simply had to maintain fixation throughout the trial. During the task, we recorded from populations of neurons from PPC and PFC using two 32-channel semi-chronic microdrive systems (Gray Matter Research, MT). Across 36 sessions from two monkeys, we recorded from over 350 PPC and 500 PFC isolated single units. Linear classification analysis of the neural population response reveals that spatial information first develops within 100 ms of flash onset in areas ventral to the principal sulcus in PFC, before spreading to areas dorsal to the principal sulcus and to the PPC. Classification accuracy was significantly lower for both PFC and PPC neural responses during the flash mapping task, suggesting that spatial encoding in these areas is context dependent. Further analysis will focus on the putative mechanisms that underlie the transformation of spatial information into a categorical decision.

Disclosures: **N.Y. Masse:** None. **J.M. Hodnefield:** None. **D.J. Freedman:** None.

Poster

060. Visual cognition: Decision Making

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Title: Neurons in the lateral prefrontal cortex compensate for the asymmetries of their sensory inputs during memory-guided comparisons of visual motion

Authors: *K. MICHALOPOULOS[1,2], P. M. SPINELLI[1,2], T. PASTERNAK[1,2];
[1]Dept. of Neurobio. and Anat., [2]Ctr. for Visual Sci., Univ. of Rochester, Rochester, NY

Abstract: Comparing two visual motion stimuli that occur at different times require processing and storage of the initial stimulus, followed by its retrieval and comparison to the current stimulus. Such tasks demand coordination of processes involving bottom-up sensory information and top-down cognitive signals. This coordination is likely to take place in the lateral prefrontal cortex (LPFC) since it receives inputs from the motion processing area MT and its activity reflects task engagement and participation in sensory maintenance. When motion comparisons involve stimuli presented at the fovea, neurons in the LPFC show direction selective (DS) responses indicative of their MT origins and display anticipatory and memory-related activity, the likely components of its top-down influences. Little is known about LPFC activity during tasks involving motion presented outside the fovea. This question is of interest because of the connectivity between LPFC and the highly retinotopic area MT: while the information about the contralateral motion can be supplied directly by MT in the same hemisphere, the ipsilateral motion represented by MT in the opposite hemisphere, can only reach LPFC indirectly, most likely via callosal connections from the opposite LPFC. We examined how the LPFC neurons represent and utilize motion information that originates in the ipsilateral and contralateral hemifields while monkeys compared directions of two stimuli, S1 and S2, separated by a delay. During S1, responses to the contralateral motion were stronger and preceded ipsilateral responses by ~40ms, an indication of the apparent dominance of direct inputs from the ipsilateral MT. The asymmetry between contralateral and ipsilateral responses during S1 was not reflected in their DS activity, since it was equally robust for both stimulus locations. During S2, responses to ipsilateral but not contralateral motion were enhanced, eliminating the dominance of the contralateral stimuli observed during S1. In addition, the activity of many neurons during and after S2 presented in either of the two hemifields was modulated by the direction of S1, reflecting the difference between current and remembered stimuli and was predictive of the perceptual report. These results demonstrate that the process of memory-guided stimulus comparisons seamlessly incorporates sensory information from both hemispheres. Thus, while the sensory components of the LPFC activity reflect the difference between their direct and indirect origins, it appears that its cognitive components can compensate for the inequalities in their bottom-up sensory signals.

Disclosures: **K. Michalopoulos:** None. **P.M. Spinelli:** None. **T. Pasternak:** None.

Poster

060. Visual cognition: Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: D.04. Vision

Title: Mechanisms of perceptual learning and learning interference in visual categorization

Authors: *L. SHA, R. KIANI;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Practice is the basis of visual expertise; it improves the speed and accuracy of acting on visual information. This improvement, known as perceptual learning, could stem from different mechanisms: (i) better representation of visual features, (ii) improved interpretation (i.e., optimal weighting) of visual information for decision-making, or (iii) improved decision strategies for committing to appropriate choices. While changes in the representation and weighting are well established, changes in decision strategy remain underexplored. Here, we investigate all three mechanisms. Six human subjects categorized multi-feature silhouettes with one informative feature whose attributes (e.g., width, height, and polarity) were quantitatively modified from trial to trial. The category boundary was defined on multiple attributes, forming a slanted hyperplane in the attribute space. Learning occurred based on distinct auditory feedbacks for correct and incorrect choices. We recorded changes in accuracy and reaction times (RT) to quantify learning. Accuracy improved with practice, ultimately shaping well-defined psychometric functions with monotonically increasing accuracy as a function of distance from the category boundary. RTs changed correspondingly; they decreased for the far stimuli and increased for those near the category boundary. Following training, we tested learning interference by introducing a second block, where subjects learned new categories defined either by rotating the hyperplane of category boundary in the same attribute space (similar condition) or by changing the informative attributes and features (dissimilar condition). Then, subjects were retested for the categorization of the first block. Accuracy significantly diminished and subjects took more trials to recover following similar blocks, demonstrating learning interference. Using decision-making models and psychophysical kernel analyses, we show that behavioral changes in perceptual learning result from (i) increased sensitivity to visual attributes, (ii) optimal weighting of the attributes to match the imposed category boundary, and (iii) increased decision criterion to adjust speed-accuracy tradeoff. Our results indicate that, in addition to changes in the representation and interpretation of sensory information, adjustments of decision strategy account for a sizeable portion of learning effects. On the other hand, learning interference was chiefly manifested by suboptimal weighting of feature attributes, suggesting that visual sensitivity and decision strategy can be preserved across interfering learning episodes.

Disclosures: **L. Sha:** None. **R. Kiani:** None.

Poster

060. Visual cognition: Decision Making

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Simons Collaboration on the Global Brain Postdoctoral Fellowship

Title: Post-error adjustments of decision strategy are informed by decision confidence

Authors: *B. PURCELL, R. KIANI;
New York Univ., New York, NY

Abstract: The ability to flexibly adjust our decision strategy following negative outcomes is vital to survival. After negative feedback, humans often slow down to respond on subsequent decisions. This post-error slowing (PES) prevents additional errors at the cost of deliberation time. Here, we directly test the hypothesis that PES is shaped not solely by negative feedback, but by a comparison of the expected and actual outcomes of past decisions. An unanticipated negative feedback indicates that the decision-making process is not appropriately tuned to the situation and calls for a revision. We measured post-error changes in the behavior of humans performing a reaction time version of a direction-discrimination task with stochastic moving dots. We manipulated task difficulty by randomly varying the motion strength (percentage of coherently moving dots) across trials. Subjects simultaneously reported the net direction of motion (up or down) and their confidence by making a saccadic eye movement to one of two possible targets above or below the dot patch (Kiani et al., 2014). The targets were elongated horizontal bars and the landing point of the saccade on the target indicated confidence. We found that post-error adjustments in decision-making were highly dependent on subjects' expectations. Errors associated with higher confidence produced large elevations of reaction times on the next trial, especially for weaker motion strengths. In contrast, PES was substantially reduced following lower-confidence errors. In both conditions the overall accuracy and psychophysical thresholds remained unchanged. The observed pattern is best explained through a mixture of increased decision bound and decreased sensitivity to incoming evidence. These results challenge the proposal that negative feedback drives PES through purely adaptive changes in decision bound or purely maladaptive distraction effects. Instead, the magnitude of mismatch between actual and expected outcomes drives changes in the stimulus sensitivity and decision strategy, which determine the magnitude of PES.

Disclosures: **B. Purcell:** None. **R. Kiani:** None.

Poster

060. Visual cognition: Decision Making

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Topic: D.04. Vision

Support: ERC Parietalaction

Title: Making decisions about observed actions

Authors: *A. PLATONOV;
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Abstract: Despite significant progress in identifying brain circuit for processing action observation, there is no general framework to establish a relationship between this brain activity and its behavioural correlates. We propose that varying the amount of dynamic noise in action movies as done previously in random dot motion direction discrimination studies, provides such paradigm. In 4 experiments 10 human subjects discriminated between two manipulative hand-actions in a 2AFC. In experiments 1-3, observers were presented with rolling/rotation movies, in experiment 4 with a dragging/grasping pair. In each movie a male or female actor manipulated one of two objects. Actions were presented at 2 depths and 5 fronto-parallel positions, resulting in 40 movies per action. On every frame in each movie, a percentage of randomly chosen dot-pixel pairs separated by a distance randomly defined from within a fixed interval was scrambled. By manipulating this percentage, we set signal strength at different levels from nearly 0 to 100%. In experiment 1, observers reacted at the end of a trial. For each full action movie, we created two additional copies retaining only the static or dynamic component. Accuracy changed as a function of stimulus strength following a classical psychometric function. Neither static nor dynamic component alone could account for these results. In experiment 2, 3 and 4, the subjects watched full action movies and responded as soon as they made a decision. Different observers took part in experiment 2 and 3, and all accomplished the same amount of full action movie blocks in the training prior to testing. Observers in experiment 2 discriminated between 2 actions while, in experiment 3, action discrimination blocks were randomly intermingled with blocks in which subjects discriminated gender (male/female). The response time and accuracy were closely coupled in all three experiments. The data were well fit by a proportional rate diffusion model with 3 parameters (bound, drift rate and residual time), the threshold ratio being close to 3.5 (characteristic for diffusion models). Changing task in experiment 3 significantly affected drift rate, a factor 3 increase in gender discrimination revealing a faster evidence accumulation rate for the same visual input. In experiment 4, as the observers received no preliminary training,

their data suggest that learning templates for new actions of the same class play very little role in action discrimination. Our results posit action observation as an ethologically valid extension of motion direction discrimination for decision making studies.

Disclosures: A. Platonov: None.

Poster

060. Visual cognition: Decision Making

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Support: NSF BCS-1358955

Title: Dissociable adjustments at abstract accumulator and motor preparation levels in different urgency regimes

Authors: *N. A. STEINEMANN[1], R. G. O'CONNELL[2], S. P. KELLY[1,3];
[1]Biomed. Engin., Neural Systems Lab, City Col. of New York, New York, NY; [2]Inst. of Neurosci., Trinity Col. Dublin, Dublin, Ireland; [3]Electrical Engin., Univ. Col. Dublin, Dublin, Ireland

Abstract: The prevalent view from mathematical psychology that speed-emphasis in decision-making tasks leads to decreased decision thresholds, has recently been challenged by findings of the opposite effect in frontal eye field and lateral intraparietal area in monkeys. Employing human electroencephalography, correlates associated with decision formation, like the centro-parietal positivity (CPP) can be acquired simultaneously with signals of motor preparation. In the current study we analyze motor preparation activity in the mu band (8-14 Hz) separately over motor cortex contralateral and ipsilateral to the correct movement in order to assess the effects of urgency on the separate, competing accumulators for the two alternatives at the motor level, and compare these dynamics with those observed at the abstract accumulator level (the CPP). We employed a two alternative forced-choice contrast discrimination paradigm as well as a random dot motion (RDM) task. In both, we manipulated speed versus accuracy emphasis, and additionally in the RDM task we manipulated the level of counter-evidence, i.e., motion towards the incorrect alternative direction. In both experiments, the average CPP appeared to reach higher thresholds in the speed the the accuracy condition, but analysis of peak CPP amplitude over response time quantiles revealed that for early RTs in common with both conditions, CPP amplitude tended to be higher for the accuracy condition, and in both speed and accuracy

conditions, this amplitude-at-RT steeply decreased over increasing reaction times consistent with a collapsing bound. In contrast, contralateral motor preparation signals at response time showed a stable level across RT bins . Ipsilateral (unchosen) motor preparation, however, showed a clear increase for the speed condition, and both contralateral and ipsilateral motor preparation built more steeply, consistent with an additive urgency signal exerted at the motor level. In ongoing analyses we are testing whether models incorporating these and other features of the neurophysiological data accurately describe the behavioral data.

Disclosures: **N.A. Steinemann:** None. **R.G. O'Connell:** None. **S.P. Kelly:** None.

Poster

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Title: Laminar differences in neural activity covarying with action choice in dorsal premotor cortex

Authors: *C. CHANDRASEKARAN[1], D. PEIXOTO[2,6,3], W. T. NEWSOME[2,7], K. V. SHENOY[1,2,4,5];
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Abstract: Dorsal premotor cortex (PMd) is involved in choosing appropriate motor actions on the basis of visual cues. However, we currently do not understand how the action selection process unfolds in the underlying circuit in PMd. We addressed this question by recording with

linear multi-contact electrodes (16 contacts, 150 μm) in PMd of monkeys performing a visual discrimination reaction time (RT) task. Monkeys discriminated the dominant color in a central static checkerboard containing mixtures of isoluminant red and green squares, and then reached to either a red or green target (presented prior to the checkerboard) as the behavioral report. Changing checkerboard difficulty modulated the monkeys' accuracy and induced a range of RTs. Thus, the task induces rich behavior, which allows study of the neural correlates of this deliberative action selection process. We have previously demonstrated that a diverse PMd neural population covaries with action choice. A subpopulation of these cells had properties consistent with a candidate decision variable (Chandrasekaran et al., SFN 13, 14). Here we report physiological data from 416 single and 131 multi-units in monkey T PMd (385 from U-probes). The PMd units fell into two broadly overlapping classes. The first class of cells modulated their responses with checkerboard onset;the response duration covaried with RT. The other class is perimovement cells that respond around movement onset (104 units, 17%). The first class can be subdivided into cells, which on checkerboard onset either suppress (105, 20%) or enhance their firing rates (348, 63%). The responses of enhanced cells were consistent with a candidate decision variable. We captured this diversity using a visuomotor index. To test for an organization as a function of depth, we related the visuomotor index to the electrode contact at which the unit was recorded; contact 1 was superficial and 16 the deepest. We pooled across days to identify distributions of contact indices for each cell class. Although pooling likely reduced depth differences, enhanced units were more likely in superficial electrodes when compared to suppressed (7 vs 11, $p < .001$) and perimovement units (7 vs 10, $p<.001$). Perimovement and suppressed units were not found at different depths ($p > .05$). Consistent with this finding, the visuomotor index decreased with increases in depth ($r=-0.66$, $p<.01$). Finally, on average, choice predictive activity was present at least 27 ms ($p<.001$) earlier in superficial electrodes (1-6) than deeper electrodes (10-14). This basic statistical analysis suggests a depth, and thus laminar, organization of functional neural populations in PMd mediating action choice.

Disclosures: **C. Chandrasekaran:** None. **D. Peixoto:** None. **W.T. Newsome:** None. **K.V. Shenoy:** None.

Poster

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Burroughs Wellcome Fund Career Award

Howard Hughes Medical Institute

Fundacao para a Ciencia e Tecnologia

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Title: Dorsal premotor cortex reflects decision making only when concrete actions are available

Authors: *M. WANG[1], C. CHANDRASEKARAN[2], D. PEIXOTO[3,1,5], W. T. NEWSOME[3,6], K. V. SHENOY[2,3,1,4];
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Abstract: Lesion and physiological studies suggest that dorsal premotor cortex (PMd) is involved in decisions based on visual cues. We previously demonstrated that subpopulations of PMd neurons may reflect a candidate decision variable in decision making tasks (Chandrasekaran et al., Peixoto et al. SFN 13, 14). However, in these studies, the perceptual decisions were explicitly linked to real or potential action plans. Here we focused on dissecting the degree to which neural dynamics in PMd reflect the processes of perceptual decision making and action selection. We trained monkey T to reach to a target whose color matched the dominant color in a central red-green checkerboard. To dissociate between perceptual decision (regarding the dominant color) and action selection (reach to either the left or right target), we used two task design manipulations. First, on a trial-by-trial basis we randomized the target configuration so that on some trials, the left target was red and the right target was green, and vice versa. Second, we used two types of trials which were interleaved. One was the classic design in which targets appeared first, followed by the checkerboard ("TargFirst"). In the new design, the presentation was reversed, so the checkerboard appeared first, followed by the targets ("StimFirst"). In the time period after the checkerboard is presented and the targets have yet to appear, there is not enough information to form an action plan because the target configuration is randomized. Psychophysical thresholds were largely similar in the two conditions (signtest, $p = 0.16$). We recorded from 33 units in PMd (9 single units, 24 multiunits; single electrodes). Consistent with our previous reports, in the TargFirst condition, PMd did not respond after target onset, and had properties consistent with a candidate decision variable after checkerboard onset. In contrast, in the StimFirst condition, we found the reverse: PMd did not respond after checkerboard onset ($p > 0.05$), but did become choice predictive in a lawful manner after target onset. Stated differently, information from the targets and from the checkerboard were each necessary, but not sufficient, to modulate PMd neurons; only when both types of information were available did PMd activity change. Several studies have suggested that both premotor and

parietal areas are involved in decision making. By using task designs that separate perceptual decisions from action selection, we suggest that PMd is engaged only when concrete actions are available and it is not involved in forming purely perceptual decisions. Thus, unlike parietal areas, neural dynamics in PMd might be more tightly linked to action selection processes.

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Poster

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Support: HHMI

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NIH EY011378

Title: What does LIP do when a decision is dissociated from planning the next eye movement?

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Abstract: Neurons in the lateral parietal area (LIP) reflect the accumulation of evidence when a decision is communicated by an eye movement into, or away from, a neuron's response field (RF). In most experiments, a saccadic choice follows the decision immediately or after a memory delay. However, not all decisions precipitate an immediate action. In fact, under conditions requiring a sequence of eye movements, LIP neurons represent only the next eye movement (Mazzoni et al. 1996). Therefore, when a decision is not communicated with the next eye movement, LIP might not reflect the decision formation but instead represent only the next eye movement. We trained a rhesus monkey to decide the net direction of motion in a stochastic random dot display. Although the monkey ultimately indicated its choice with a saccadic eye movement, it first made a sequence of irrelevant eye movements, away from and back to the original point of fixation. The intervening steps did not impair the monkey's performance on the discrimination task. We examined the response of LIP neurons during decision formation with and without a choice target in the RF. Despite the fact that the next eye movement was always to

an irrelevant target outside the neuron's RF, LIP activity reflected the accumulation of evidence supporting the ultimate saccadic choice towards the neuron's RF. After decision formation, as the intervening saccade displaced the choice target from the neuron's RF, these neurons no longer represented the choice information. Instead, the information was represented by other LIP neurons as the irrelevant eye movements brought a choice target into their RF. Then, with acquisition of the original fixation, the LIP neurons that reflected decision formation were reanimated to represent the choice information. The restored choice-related activity was correlated with the decision-related activity the same neuron represented during decision formation, despite the intervening eye movements. Thus, even when a decision is to be reported after irrelevant eye movements, LIP neurons exhibit decision-related activity, and they do so again around the time of the ultimate, relevant eye movement to report the choice. During the intervening eye movements, the choice information is represented in other LIP neurons with different RF locations. Together, these results expose an unexpected capacity of LIP neurons, as a population, to mediate a decision-related computation spanning multiple oculomotor actions.

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Poster

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Radboud University Nijmegen Medical Centre

Title: Primate saccade target selection relies on feedback competitive signal integration

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Abstract: It is often assumed that decision-making involves neural competition, accumulation of evidence 'scores' over time, and commitment to a particular alternative once its 'scores' reach decision-threshold first. So far, however, neither the first-to-threshold rule, nor the nature of competition (feed-forward or feedback inhibition), has been revealed by experiments. Here, we

presented two simultaneously-flashed targets that reversed their intensity difference during presentation, and instructed subjects to saccade towards the brightest target. Both humans (n=6) and monkeys (n=2) preferentially chose the target that was brightest during the first stimulus phase. Unless this first phase was too short, primacy persisted even if the second, reversed-intensity phase lasted longer. This effect did not result from premature commitment to the initially-dominant target; a strong target imbalance in the opposite direction later on drove nearly all responses towards that location. Moreover, there was a non-monotonic relation between primacy and target imbalance; increasing this imbalance beyond 40 cd/m2 caused an attenuation of primacy. These are the hallmarks of hysteresis, predicted by models in which target-representations compete through strong feedback. Preliminary analysis indicates that this behavior is reflected in the single-unit activity of cells in the Frontal Eye Fields.

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Poster

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Title: Monkeys behaving badly: probing macaque subjects' internal task strategies with psychophysical reverse correlation

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Abstract: Our understanding of the neurobiology of perceptual decision making relies heavily on studies where animal subjects perform threshold psychophysics. A fundamental premise is that the behavioral relevance of sensory evidence is under the experimenter’s control, typically examined with threshold measures. However, these provide no guarantee that animals correctly perform the task assigned. Understanding the rules used by animals to render perceptual reports is critical to the interpretation of many studies. We used psychophysical reverse correlation to measure macaque subjects’ internal task strategies, in a coarse orientation discrimination task. Animals reported the orientation of a stimulus from two orthogonal alternatives, which were

fixed within a session. The stimuli consisted of dynamic white noise filtered in the Fourier domain. The filter's center orientation varied between the two discriminanda, and its angular width controlled task difficulty. Some trials were filtered with an infinitely broad angular component, thus containing a uniform distribution of orientations on average. The animal's decision rule (or "psychophysical kernel") was calculated as the difference between the two choice-conditioned mean orientation power spectra of these trials. The orientation of this kernel describes which stimulus orientations most strongly influenced the animals' choices. The kernel orientation frequently deviated from the discriminanda, providing objective evidence that the animals did not do the task as assigned. The magnitude of deviation predicted the day to day variability in behavioral threshold (r=0.32). To explore the effect of recent training history, we kept the discriminanda fixed for multiple days. Following a change in the discriminanda, the kernels initially reflected the previous task. Remarkably, the time constant for aligning the kernel to the new task was approximately 10 training days (50,000 trials). In striking contrast, human subjects were able to switch their decision rule within a single session. The accuracy of the kernel in predicting individual choices (approximately 52% on average) provides an estimate of internal noise. If animals treat all trials equally (as they should if they are at threshold), this estimate should not change with signal strength, providing a useful metric of whether subjects are performing at threshold. Psychophysical reverse correlation provides powerful tools to assess whether animal subjects do the task they have been set. This information is essential for interpreting many studies, especially those employing dynamic changes in task.

Disclosures: **A.G. Bondy:** None. **B.G. Cumming:** None.

Poster

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Title: Uncertainty decoded from population activity in macaque primary visual cortex is used in perceptual decisions

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Abstract: Organisms typically base their perceptual decisions on noisy and ambiguous sensory observations. There can be top-down sensory uncertainty due to ambiguity and bottom-up sensory uncertainty due to noise in the perception system. In many tasks, optimal performance requires the brain to represent and utilize, on every trial, knowledge about the level of both bottom-up and top-down uncertainty. In earlier work, we introduced a simple orientation classification task with controlled top-down and bottom-up sensory uncertainty for which optimal performance requires the observer to utilize sensory uncertainty on a trial-by-trial basis, and demonstrated that both humans and monkeys do so. Here, our goal is to identify the neural substrates of this computation. The theoretical framework of probabilistic population coding (PPC) postulates that the brain decodes sensory uncertainty from a noisy pattern of population activity through a "likelihood function" over the stimulus. This function represents the probability of the observed pattern given each hypothesized stimulus value, and the width of this function is a proxy of sensory uncertainty. We hypothesized that the width of the likelihood function that can be decoded from trial-to-trial population activity in primary visual cortex (V1) is informative about the animal's decision. To test this hypothesis, we trained macaque monkey on our classification task. We implanted a chronic multi-electrode array in V1 to record the population activity while the monkey performed the classification task. On each trial, we decoded from single-trial V1 population activity the width of the likelihood function under a Poisson-like population coding model. The monkey's trial-by-trial classification decisions were better predicted by a Bayesian model utilizing the width of the likelihood function than by a non-Bayesian model only utilizing a point estimate of the stimulus orientation. We also tested the models on a shuffled data where the widths of the likelihood functions were shuffled among trials with identical stimulus condition, effectively removing trial-by-trial correlation between the likelihood width and the monkey's decision, while keeping the average correlation between likelihood widths and stimulus orientations intact. We observed that Bayesian model's performance dropped significantly on the shuffled data when compared to the fits on the original data, supporting our hypothesis that trial-by-trial variation in the likelihood width is informative about the decision. This result provides population-level physiological evidence in support of the PPC framework.

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Poster

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Support: Netherlands Organization for Scientific Research NWO 404-10-037

Title: Serial dependence in visual perception and stimulus representation in primary visual cortex

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Abstract: Our perception of the world is partly determined by our knowledge of the statistical regularities in the environment, such as the fact that the world is stable over short time scales. This is reflected by the fact that visual perception is serially dependent, with previously seen stimuli biasing the perception of subsequent stimuli (Fischer & Whitney, 2014). The neural mechanisms of this effect are unclear. Does this trial history effect occur at the level of the higher-order areas involved in perceptual decision making (Gold & Shadlen, 2007), or do preceding stimuli bias stimulus representations at the earliest levels of sensory processing? In this study, we acquired fMRI data in 24 healthy human participants while two grating stimuli were briefly presented in the left and right visual field. On each trial, participants reported the orientation of the grating at the location indicated by a post-stimulus response cue. We used multivariate pattern analysis to extract orientation specific BOLD signals from the primary visual cortex on every trial. We applied multiple regression analyses to isolate the influence of the current and previous stimuli on the behavioural and neural responses. In line with previous reports (Fischer & Whitney, 2014; Liberman, Fischer, & Whitney, 2014), perceived orientation was consistently biased towards the orientation of the preceding stimulus. Strikingly, the orientation signal in V1 was similarly biased towards the orientation presented on the previous trial, suggesting that recently seen stimuli alter low-level sensory representations. Serial dependence was spatially specific such that stimuli on the current trial only showed an influence of previous stimuli at the same location, both in terms of brain and behavior. Finally, when stimulus and perceptual choice diverged, trials were biased by previous choice, rather than previous stimulus. Our study extends previous behavioral reports by revealing a trial history effect at the earliest levels of sensory cortex. This biasing process is spatially specific, and governed by previous choice rather than stimulus. Together, these findings elucidate how our perception is shaped by our perceptual history.

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Poster

060. Visual cognition: Decision Making

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Sciex 13.065

Title: The interplay between long-and short-term memory traces in sequential visual decision making

Authors: *J. FISER, J. ARATO, A. KOBLINGER;
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Abstract: Past experience strongly guides sensory processing and influences every perceptual decision. Yet, due to contradictory findings in the literature, the exact pattern of these effects is unclear and a convincing general computational framework underlying these effects is still missing. Even in the simplest version of the problem, making a forced choice between two hypotheses based on noisy sequential input, the field is divided over how basic statistics of the input (e.g. appearance frequencies) and various significant patterns (e.g. repetition) jointly determine the observer's behavior. We used the above model problem in 7 experiments to tease apart the relative contributions of each effect on human sequential decision making. Observers performed a 2-AFC decision making ("Which of the two shapes is seen?"), while we independently modulated the level of pixel-noise, the appearance frequency of the elements coming from the two classes at two different time scales, and the ratio of repetition/alternation in the sequence. We found that the noise level of the stimulus systematically modulated the strength of each contributing factor to decision making. However, instead of a simple interpolation between long-term probabilities and veridical choice as it would be predicted by adaptation or priming, different pairings of short- and long-term appearance probabilities produced various characteristic under- and over-shootings in choice performances. This rules out a number of earlier models proposed for explaining human behavior in such tasks. We also found that human performance measured by correct answers and by reaction times yielded opposing results under some conditions indicating that RT measures tap into motor rather than cognitive components of sequence coding. By controlling the base-rate probabilities and repetitions/alternations independently, we also observed that despite the two measures being correlated in general,

repetition/alternation is a factor independently influencing human judgment. To assess the generality of our findings, we run behavioral studies with adult rats asking them to choose between two full-field stimuli of different brightness. We found that rats replicated the striking results of humans, by choosing the frequent stimulus of recent past fewer times under high uncertainty after experience with particular long-term appearance statistics. Through simulations, we confirmed that our results can be captured by a probabilistic model of human visual decision making that balances long- and short-term summary statistics of sequences, and in parallel, also encodes salient features, such as repetitions in the sequence.

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Poster

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Title: Dissociating sensory from decision processes in human perceptual decision making

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Abstract: Theoretical frameworks posit that perceptual decisions arise from a cascade of functionally distinct stages. In the sensory stage, the physical stimulus is encoded into internal sensory evidence, while during the subsequent decision stage, this sensory evidence is integrated over time into a decision variable. Identifying and dissociating these stages at the neural level will advance our understanding of perceptual decision making and has become a central goal within the field. We adopted a novel approach to dissociate sensory- from decision-related activity in MEG, while subjects were making perceptual decisions about the presence/absence of a grating embedded in noise. We used a functional localizer, in which subjects were exposed to grating stimuli while performing a task at fixation, in order to isolate neural signals related to sensory processing in the absence of decision making and attention. A multivariate decoder was trained on this data to identify a sensory-specific neural signature. We subsequently employed this decoder to trace sensory processing over time during perceptual decision making. In addition, we conducted an analysis in which we trained a decoder on the data obtained during the perceptual decision making task itself, without making use of the functional localizer, to extract

the neural signal that collectively underlies sensory processing and decision making. The results showed that sensory-related activity was specific to early time windows and consistent with occipital sources. Interestingly, we observed that the stimulus information was sustained when it was attended and relevant for the task at hand. Furthermore, the stimulus information faithfully reflected the physical stimulus, regardless of the eventual behavioral decision. Decision-related activity, on the other hand, was longer-lasting and more prominent during later time windows. It showed a ramping temporal profile and was localized to parietal and frontal cortex. Furthermore, decision-related activity was confined to a later time window when no grating was presented, but included the early time points when a grating was presented - the time window that was previously associated with sensory processing. We suggest that this early decision-related activity may reflect fluctuations in latent confounding variables, such as attention. In conclusion, our approach provides a novel way to reliably extract the neural dynamics of sensory processing during perceptual decision making, uncontaminated by decision processes or other confounding variables.

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Poster

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Title: Eye-opener: pupil dilation signals decision uncertainty

Authors: *A. E. URAI[1,2], J. W. DE GEE[1,2], T. H. DONNER[2,3];
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Abstract: Purpose: Neuromodulators released from ascending brainstem systems gate cortical plasticity and perceptual learning, and have been implicated in signaling uncertainty during

decision-making. During perceptual decisions, observers' certainty about successful decision outcomes increases monotonically with the strength of sensory evidence on correct trials, but follows the opposite pattern on error trials: if sensory evidence is strong but the observer chooses wrongly, the internal decision variable is more likely to be close to the decision criterion (leading to larger uncertainty). Here, we used pupil dilation at constant luminance as a proxy for neuromodulatory brain state. During a protracted perceptual decision with feedback, we systematically manipulated decision uncertainty. We asked how, and at which time during the trial, this manipulation modulates the amplitude of pupil dilation. **Methods** We measured pupil diameter in 27 human observers performing a 2-interval forced choice task. Dynamic random dot patterns with varying motion coherence were shown in two successive intervals, and observers judged which interval contained stronger motion. The strength of sensory evidence (i.e., coherence difference between intervals) varied from trial to trial. After ~2 s following response, observers received auditory positive or negative feedback. **Results** As predicted, reaction time, a proxy of decision uncertainty, decreased with sensory evidence on correct trials, but increased with sensory evidence on error trials. Pupil dilation during decision formation scaled with sensory evidence, and hence certainty, in this same way. Pupil dilation after feedback, on the other hand, reflected decision uncertainty only on correct trials. Following negative feedback, the pupil dilated robustly, but dilation amplitude was unrelated to sensory evidence. Applying a reinforcement learning model to our data, preliminary results indicate that observers, whose decision-linked pupil dilation most precisely tracks uncertainty across trials, show higher learning rates. **Conclusions** Pupil dilation during protracted perceptual decisions reflects decision uncertainty. The precision of this uncertainty coding might drive perceptual learning. After feedback, the pupil reflects prediction errors (i.e., the difference between decision certainty and received feedback) only after positive, not negative, feedback. Ongoing pharmacological interventions during magnetoencephalography and pupillometry recordings aim to uncover neuromodulation-induced changes in cortical dynamics during perceptual learning.

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Poster

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Amsterdam Brain and Cognition center (ABC2014-01)

Title: Decision-related pupil dilation reflects locus coeruleus activity and altered visual evidence accumulation

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Abstract: Purpose The brainstem locus coeruleus (LC) sends widespread modulatory (noradrenergic) projections to the cortex (including visual and parietal cortex), and it is connected with other neuromodulatory brainstem centers. In monkeys, the LC exhibits transient activations during visual decisions. These activations are thought to be caused by top-down input from frontal cortical regions (e.g., anterior cingulate cortex, ACC) and to drive pupil dilation. We used high-resolution fMRI to track transient LC responses in humans during a visual yes-no contrast detection task. We tested for the link between these LC responses and (i) decision-related (non-luminance mediated) pupil dilation; (ii) decision-related responses in dopaminergic midbrain centers (substantia nigra, SN; ventral tegmental area, VTA), and the cholinergic basal forebrain (BF); and (iii) the computations leading to a simple decision, such as the accumulation of sensory evidence. **Methods** Human subjects (N=15) performed a near-threshold contrast detection task during concurrent whole-brain fMRI (1.79x1.79x3.0 mm) and pupillometry. They viewed a continuous stream of dynamic black and white noise centered around the fixation mark on a grey background. Each trial started with an auditory cue, followed by, in 50% of trials, the superposition of a low-contrast target grating onto the noise. Subjects reported their yes-no judgment about target presence by a button press. To quantify the effect of pupil-linked neuromodulation on the protracted decision process (median RT: 2.08 s), we fitted a sequential sampling (drift diffusion) model to the reaction time distributions for yes and no choices, separately for high and low pupil dilation trials. **Results** The amplitudes of decision-related LC responses correlated with pupil dilation amplitude on a trial-by-trial basis. Significant pupil-coupling was also evident for SN/VTA (not BF), but this effect did not explain the LC-pupil coupling. Decision-related LC responses were also correlated with responses in the ACC, the putative driver of decision-related LC activation. Drift diffusion modeling revealed a selective bias of the accumulation ("drift criterion" parameter) towards yes-choices under high pupil dilation. **Conclusion** Decision-related pupil dilation reflects LC responses. These, in turn, cause a transient boost in neuromodulation in cortical networks that pushes the accumulation of sensory evidence during yes-no decisions toward "yes".

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Title: Decision-related oscillatory activity in human visual cortex is linked to pupil dilation

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Abstract: Introduction Behavioral report of the disappearances of salient visual targets is accompanied by a transient, retinotopically global modulation of population activity in human visual cortex. This signal is contingent on behavioral report of the (illusory or physical) target disappearance and its strength predicts the stability of the subsequent perceptual state. We used magnetoencephalography (MEG) to test if this novel top-down signal is associated with (i) the perceptual decision or the resulting motor response, and (ii) a transient boost of neuromodulation. We used pupil dilation at constant luminance as a non-invasive proxy of central neuromodulator release. **Methods** We performed simultaneous MEG and pupil diameter recordings in N=28 human subjects during the spontaneous ("motion-induced blindness" illusion) or physical temporary disappearances of a salient visual target (full contrast Gabor, size: 2 deg, spatial frequency: 2.5 deg^{-1}) surrounded by a moving flow field. Subjects reported these perceptual changes by overt motor response (button press), or covert counting (report of total after run). We characterized the effects of (i) motor report and (ii) pupil dilation amplitude on the modulations of MEG power over visual cortex around perceptual changes. **Results** As in earlier studies, a transient modulation of MEG power in the beta frequency range (12-36 Hz) over visual cortex reflected the content of behavioral report (suppression for target disappearance, enhancement for reappearance). An analogous modulation occurred in the alpha range (8-12 Hz). Both modulations occurred at the median response time (from the overt report condition) during both overt report and covert counting. The modulations were enhanced under high pupil dilation.

Conclusions The power modulation in visual cortex reflects a top-down signal linked to the perceptual decision, irrespective of the overt motor action. This, together with the boost under increased pupil dilation, is consistent with an origin in neuromodulatory brainstem centers. Ongoing analyses aim at quantifying the effect of Bayesian surprise, a putative driver of phasic responses in these centers, on the decision-related top-down signal in visual cortex.

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Poster

060. Visual cognition: Decision Making

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Deutsche Forschungsgemeinschaft (DFG), SFB 936

Title: Effects of noradrenaline on visual evidence accumulation in human cortex

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Abstract: Purpose: Perceptual decision-making entails the accumulation of sensory evidence towards thresholds for alternative choices. This accumulation results from recurrent interactions in networks of association cortex producing persistent activity. Recurrent cortical interactions are shaped by the catecholamine noradrenaline (NA): NA boosts persistent activity and dampens trial-to-trial variability of cortical neurons. However, it is unknown how NA shapes the cortical network dynamics underlying perceptual decisions. Here, we manipulated central NA levels with atomoxetine (selective NA reuptake inhibitor) and characterized the effects on the dynamics of an elementary visual decision by magnetoencephalography (MEG) and computational (drift diffusion) modeling of choice behavior. **Methods:** Human subjects (N=18) performed a spatial 2AFC discrimination task, comparing the contrast of two Gabor patches (diameter 20°) in the

lower left/right visual field. Subjects reported the location of the higher contrast ("target") by left or right hand button press (variable stimulus-response mapping, free response paradigm). Two contrast differences were titrated to the individual 85% ("easy") or 70% ("hard") correct levels. Each subject performed four experimental sessions, with administration of atomoxetine or placebo (randomized, double-blind, within-subjects design). We fitted the drift diffusion model to the reaction time distributions (separately for drug conditions) and performed time-frequency analysis on the MEG to assess the effects of drug on frequency-specific modulations of the cortical power spectrum during decision formation. **Results:** Compared to placebo, atomoxetine enhanced beta-band (12-36 Hz) power over occipital and parietal cortex from ~0.5 s after stimulus onset until after the response (median RT: 0.96 s). Atomoxetine also enhanced gamma-band (50-60 Hz) power over a range of regions including frontal cortex. Both drug effects were spatially global, riding on top of selective modulations that were lateralized with respect to target or response. Drift diffusion modeling showed a selective reduction of the trial-to-trial variability of drift rate under atomoxetine. This effect was highly significant when only drift rate and drift rate variability were free to vary with drug condition. It was also significant and selective in a model with four model parameters free to vary. **Conclusion:** Boosting central NA levels alters cortical network dynamics in the beta- and gamma-bands during decision formation and reduces the trial-to-trial variability of visual evidence accumulation.

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Poster

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Title: Circuit mechanisms underlying visual responses of the anterior cingulate cortex

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Abstract: Neural dynamics in sensory cortices are shaped by bottom-up inputs relaying the physical features of sensory stimuli and by top-down projections that modulate their encoding. The anterior cingulate division of the prefrontal cortex is known to provide top-down input to the visual cortex. Here, we use multiple approaches to delineate the functional role of visual inputs to the anterior cingulate cortex (ACC) and of the feedback from ACC to V1. Using rabies virus-mediated anatomical tracing to identify sources of inputs to the ACC, we found that V1 as well as other cortical and subcortical brain regions project to the ACC. Using rabies viruses that express the genetically encodable calcium indicator GCaMP6f and two-photon microscopy, we characterized the functional properties of ACC-projecting visual cortex neurons in passively viewing, awake head-fixed mice. We found that many of these neurons are tuned to the orientation and direction of drifting gratings. Next, we expressed GCaMP6s in the ACC and imaged the calcium activity of ACC axons found in layer 1 of V1. A subset of ACC axons were visually driven and displayed sharply tuned responses to the orientation and direction of drifting gratings. To assess the contribution of V1 to this property, we used a chemogenetic approach. We expressed the inhibitory hM4Di DREADD (designer receptors exclusively activated by designer drugs) in V1, GCaMP6s in the ACC, and monitored the calcium responses of ACC axons to oriented drifting gratings before and after systemic application of the DREADD agonist clozapine-N-oxide (CNO). While CNO application in control animals had no effect on the visual responses of ACC axons, it reduced responses in DREADD expressing animals. Together, these findings show that a projection from the visual cortex contributes to the visual responsiveness of the ACC. Since the ACC has been proposed to play a crucial role in cognition, and in particular reward processing, we propose that the ACC processes visual information in the context of its behavioral significance and relays a saliency signal back to the visual cortex to modulate the encoding of relevant visual stimuli.

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Poster

061. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: Mission Connect, a program of TIRR Foundation

Title: Characteristics of eye-position gain field populations in AIT and LIP determined through genetic algorithm modeling of monkey data

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Abstract: We have previously demonstrated (Sereno et al. 2014) differences between eye-position spatial maps in lateral intraparietal cortex (LIP) and anterior inferotemporal cortex (AIT) of macaque monkeys, based on population decoding of gaze angle modulations of visual responses. The LIP spatial map was approximately veridical to physical space while the AIT map was highly distorted with compression towards central fixation. Here, using a genetic algorithm, we quantify statistical differences in gain field population characteristics underlying those different spatial maps. We created a population of model neurons whose responses were modulated by eye position to form gain fields, each neuron having a different gain field. An intrinsic decoding method, namely multidimensional scaling, was used to recover an eye-position spatial map from the model population. We then used a genetic algorithm to modify the characteristics of gain field populations until the recovered spatial maps closely matched those derived from monkey neurophysiological data. The primary differences found were that AIT gain fields on average operated on smaller spatial scales and had smaller dispersions than LIP gain fields. Thus, differences in the characteristics of gain field populations for different cortical areas may underlie differences in the representation of space.

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Poster

061. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: ANR Grant ANR-13-APPR-0008-02

Title: Anticipating a moving target: role of vision and reinforcement

Authors: *A. MONTAGNINI[1], J.-B. DAMASSE[1], L. MADELAIN[1,2], L. PERRINET[1]; [1]Inst. de Neurosciences De La Timone, Marseille, France; [2]SCALab, Univ. de Lille Nord de France, Villeneuve d'Ascq, France

Abstract: Humans are able to accurately track a moving object with a combination of saccades and smooth eye movements. These movements allow us to align and stabilize the object on the fovea, thus enabling high-resolution visual analysis. When predictive information is available about target motion, anticipatory smooth pursuit eye movements (aSPEM) are efficiently generated before target appearance, which reduce the typical sensorimotor delay between target motion onset and foveation. It is generally assumed that the role of anticipatory eye movements is to limit the behavioral impairment due to eye-to-target position and velocity mismatch. However, little is known about the actual effect of target visibility on aSPEM and on the relative plasticity of this anticipatory behavior with respect to manipulations of reinforcement of different nature (perceptual or monetary). By manipulating the probability for target motion direction we were able to bias the direction and mean velocity of aSPEM (baseline condition), as measured during a fixed duration gap before target ramp-motion onset. This suggests that probabilistic information may be used to inform the internal representation of motion prediction for the initiation of anticipatory movements. To further understand the nature of aSPEM, in the first test condition we degraded the visibility of the moving target by transiently hiding it at random intervals (cumulative target blank duration=50% of motion-ramp duration). Despite being able to accurately track (pursuit gain did not significantly differ with respect to the baseline condition), anticipatory smooth pursuit was significantly reduced, suggesting a direct role of vision for the modulation of movement anticipation. Results of this first experiment suggest that accurate vision may act as an ecological reinforcer for anticipatory eye movements: thus we further tested, with two distinct experiments based on a gaze-contingent paradigm, whether aSPEM is affected by reinforcement contingencies. First, on the ground of the measured anticipatory eye-velocity, we artificially manipulated online the discriminability of a small detail (position of a Landolt-stimulus gap) of the otherwise highly visible moving target. Second, we associated a monetary reward to a criterion-matching anticipatory velocity. We observed modulations of anticipatory velocity depending on the reinforcement contingencies in both cases. Taken together, our results suggest that anticipatory eye movements are permeable to different manipulations, in particular those affecting the behavioral consequences of pursuit, either at the purely sensory or reward related level.

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Poster

061. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: Probationary Faculty Support Award

Title: Eyes off the prize: Impact of visual discomfort in college population

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Abstract: Visual discomfort is a common condition associated with performing near work tasks such as reading or viewing computer screens. Symptoms can include headaches, eye strain, double vision, blurred vision, and sensitivity to light. Two ocular systems contribute to near work performance: the accommodative system, in which the lens of the eye thickens to keep a target in focus, and the vergence system, involving the coordination of the two eyes to keep a target centered on the retina. Disorders of both of these systems have been found to be associated with visual discomfort symptoms. The Conlon Visual Discomfort Survey (VDS) has been found to reliably be associated with accommodative insufficiency (AI) while the Convergence Insufficiency Symptom Survey (CISS) has similarly been found to be associated with convergence insufficiency (CI). Given the high degree of near work associated with student success, the prevalence of visual discomfort in the college population is of great interest and relevance. Though a few studies have assessed the prevalence of visual discomfort in the college population, few have considered a model predicting the impact of these ocular dysfunctions on academic performance. A large sample of undergraduate students participated in this study. More than 40% of participants fell into the high symptom category for the VDS, and more than 65% of participants fell into the high symptom category for the CISS. Significant correlations were observed between VDS symptom scores and cumulative GPA. These preliminary data suggest a high prevalence of visual discomfort in the university population, with a significant impact on academic performance. Subsequent multiple regression analysis revealed both VDS and CISS significantly account for a portion of the variance in GPA.

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Poster

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Title: Palisade endings are a constant feature in the extraocular muscles of frontal-eyed, but not lateral-eyed, animals

Authors: *R. BLUMER[1], B. MAURER[1], J. STREICHER[1], B. GESSBAUER[1], E. PECHRIGGL[2], M. BLUMER[2], M. A. DAVIS-LÓPEZ DE CARRIZOSA[3], A. K. HORN[4], P. J. MAY[5], R. R. DE LA CRUZ[3], A. M. PASTOR[3];
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Abstract: Proprioception from extraocular muscles (EOMs) provides the brain with eye position signals. Surprisingly, classical proprioceptors (muscle spindles and Golgi tendon organs) are absent from the EOMs of most mammals. Instead, palisade endings are a unique feature of mammalian EOMs. They consist of a dense ramification of axonal branches and nerve terminals at the tip of individual muscle fibers. For a century, palisade endings have been considered as sensory structures substituting for classical proprioceptors in EOMs. Interest in palisade endings was reignited when molecular analysis and neuronal tracing experiments determined that palisade endings are cholinergic and originate from the EOM motor nuclei. To date, palisade endings have been found in all species investigated and it has been assumed that they are a general feature of EOMs. In the present study, we used immunohistochemical techniques to test this hypothesis and extended our investigation to 11 species; some frontal-eyed (human, monkey, cat, and ferret), and others lateral-eyed (pig, sheep, rabbit, rat, mouse, gerbil and guinea pig). In all frontal-eyed species, palisade endings are a constant feature in the rectus EOMs and the medial recti always contain the highest number of palisade endings (from 36 in ferrets to 85 in humans). In the lateral-eyed animals, palisade endings are constantly found in even-toed ungulates (sheep and pigs) and in rabbits. However, their density is rather low (12 - 14 in the medial recti). In rats, palisade endings are an exceptional feature: most extraocular muscles lack them. In mice, gerbils and guinea pigs, palisade endings are completely absent. Structural analysis of palisades revealed that the number of segments and the cumulative length, area and volume covaried. These features correlated with the mass of the species, following two different

allometric relations (for lateral-eyed and frontal-eyed animals, respectively), such that the largest species had the highest structural complexity. Staining with α-bungarotoxin shows that palisade endings, with few exceptions in frontal-eyed animals, lack acetylcholine receptors. In lateral-eyed animals, palisade endings in sheep and pigs also lack acetylcholine receptors; whereas palisade endings in rabbits and in rats (when present) have such receptors. Our results demonstrate that palisade endings are not a universal feature of mammalian EOMs. So if they are proprioceptors, not all species require them. Their absence in some lateral-eyed animals suggests a role in conjugacy. In fact, since the medial rectus has highest density of palisade endings, palisade endings likely play a special role in convergence.

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Poster

061. Eye Movements and Perception

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Title: Frontal Eye Fields read out, but do not assign, priority

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Abstract: A fundamental question in sensorimotor neuroscience is how we decide where to look next. One theory posits that oculomotor circuits contain a map of top-down and bottom-up priority, and that the decision of where to look next is accomplished by reading out the peaks in this priority map. The Frontal Eye Field (FEF) has been implicated in these processes, but it is not clear whether the FEF is involved in assigning priority or simply reading it out. If it is involved in assigning priority, it should passively respond to task-relevant visual inputs prior to detection. If not, it should only encode the priority of those locations where a potential target has been differentiated from the background and tagged. This issue is complicated by the fact that FEF is typically studied using stimuli and tasks that do not accurately reflect the real world. For example, saccade targets are often flashed abruptly and against a uniform background, making

target detection trivial. By contrast, most objects in the natural world are relatively static against a cluttered background, making target detection far more difficult. On the basis of these artificial tasks, previous studies have suggested that FEF passively encodes the task-relevance of visual objects - i.e., regardless of whether the target has been detected or attended to. We tested this by recording single units in FEF while macaques performed a novel target fade-in task. Monkeys fixated centrally and covertly searched for a peripheral target (a fly) that was either abruptly flashed or gradually faded into view. They then reported awareness of the target by making a saccade to it. We also varied the complexity of the background (i.e., uniform black, phase-scrambled scene, or natural scene) to measure the impact of distracting information on behavior and FEF responses. For the scrambled and natural backgrounds, visual cells responded vigorously only when the target was flashed into the receptive field. Critically, there was no graded increase in activity during the fade-in trials, for any cell type. The exception to this was during fade-in trials with a uniform black background; in these trials, there was an abrupt visual response when the target visibility passed psychophysical threshold. This suggests that FEF does not passively represent target location or accumulate evidence for saccade selection on the basis of target relevance. That is, in order for a spatial location to be assigned priority in FEF, potential targets must be differentiated from background and recognized as such. These results imply that passive relevance-based selection of space is performed upstream of the FEF in tasks that more closely resemble natural behavior.

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Poster

061. Eye Movements and Perception

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Topic: D.06. Eye Movements

Title: Representation of visual salience by frontal eye field neurons

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Abstract: Physical salience of visual targets is a key factor in driving attention and gaze toward them. We studied how activity within the frontal eye field (FEF), a key region in control of gaze

and attention, represents stimulus salience and the degree to which this representation guides the behavior. Two static stimuli were simultaneously presented onscreen, one inside the FEF neurons' response field (Sin) and the other outside (Sout). The contrast of the Sin target was constant (50%) while the contrast of the Sout stimulus varied parametrically from 0% to 100% across trials. Increasing the contrast of the Sout stimulus suppressed the visual responses of FEF neurons, indicating visual competition within the FEF. In order to determine the spatial extent of visual competition, we changed the location of the Sout stimulus relative to the Sin stimulus. The presence of the second stimulus had a suppressive effect on the visual responses of FEF neurons, far beyond the inhibitory surround of receptive fields in visual cortices. Moreover, we found that the features of visual targets have a greater impact on saccades when the target is more salient. We used moving gratings as targets and quantified the degree to which saccade endpoints are influenced by the direction of motion of the target (visual guidance). In a free choice task the animal was asked to choose between a moving grating with 50% contrast and another moving grating with 20% or 80% contrast. We found that for saccades to the same target, visual guidance is greater when it is the more salient stimulus. FEF responses not only represent the salience of visual targets, they also reflect the dependence of gaze behavior on target salience: greater FEF activity corresponds to greater visual guidance across trials. Our results implicate the FEF activity as a neural basis for the behavioral and attentional prioritization of salient stimuli.

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Poster

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Title: Neural basis of spatial mislocalization during smooth eye-movements

Authors: ***F. BREMMER**[1], S. DOWIASCH[1], G. BLOHM[2];
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Abstract: The dependence of neuronal discharge on the position of the eyes in the orbit is a functional characteristic of many visual cortical areas of the macaque. It has been suggested that

these eye-position signals provide relevant information for a coordinate transformation of visual signals into a non-eye-centered frame of reference. This transformation could be an integral part for achieving visual perceptual stability across eye movements. Previous studies demonstrated close to veridical eye-position decoding during stable fixation as well as characteristic erroneous decoding across saccadic eye-movements. Here we aimed to decode eye-position during smooth-pursuit. We recorded neural activity in the ventral intraparietal area (VIP) of two macaque monkeys during steady fixation, saccades and smooth-pursuit. Then, we aimed to determine the temporal and spatial accuracy of eye-position as decoded from the neuronal discharges. Confirming previous results, during steady fixation the activity of the majority of neurons depended linearly on horizontal and vertical eye-position. The application of a previously introduced computational approach (isofrequency-decoding) allowed eye-position decoding with considerable accuracy during fixation. In a second step, we applied the same decoder on the activity of the same neurons as recorded during smooth-pursuit. On average, the decoded signal during smooth pursuit was ahead of the current eye position. Previous behavioral studies in humans have shown a mislocalization of briefly flashed stimuli during smooth pursuit. This localization error, however, is spatially not symmetric. Instead, it is found only in the visual hemifield ahead of the fovea. Remarkably, a model combining the constant lead of the decoded eye-position, as found in our current study, with a previously described attentional bias ahead of the pursuit target describes the asymmetric mislocalization pattern during smooth pursuit.

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Poster

061. Eye Movements and Perception

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Support: Pfizer Inc Neuroscience Research Unit

Title: Informing participants impacts minute-by-minute blink count, but not average blink rate

Authors: N. SHAAFI KABIRI[1], C. R. BROOKS[1], N. KASTHURI[1], T. COMERY[2], K. C. THOMAS[1], *P. J. FRIED[3,1];
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Abstract: Objective. The act of blinking is an integral function that aids in preservation of corneal integrity. Counting blinks in humans provides valuable physiological and behavioral data. However, awareness on the part of the subject that his or her eye blinks are being counted may impact blink rate. The goal of this study was to provide direct experimental evaluation of this hypothesis. **Methods.** Thirty adult males (27.5 ± 6.7 y, 25 right-handed) provided written consent prior to enrollment. All participants had normal vision and no known neurological disorders. The study conformed to the Declaration of Helsinki. All forms and procedures received prior approval by the Institutional Review Board at Boston University School of Medicine. Six trained raters counted blinks from videos of participants' faces during eight unique epochs, consisting of four situations (spontaneous, conversation, fixation, viewing images) and two conditions (naïve and informed). Only full occlusions of the eye where treated as blinks. For each situation, minute-by-minute blink counts were entered into linear mixed model analysis with *time* and *condition* as repeated measures. **Results.** Average inter-rater reliability was 0.97. Data from one participant was excluded for excessive blinking across all epochs. While viewing images, there was a main effect of *time*, $F(2,252) = 10.6$, $p < 0.001$, indicating blink counts increased overall with time. There was no main effect of *condition*, $F(1,28) = 0.1$, $p > 0.1$, indicating average blink rate did not vary according to whether the subject was informed that blinks were counted. There was a *time x condition* interaction between the two main factors, $F(2,252) = 3.3$, $p < 0.001$, indicating that blink rate over time was impacted by being informed that blinks were counted. Paired-sample t tests revealed that blink counts were lower in naïve vs. informed conditions during minute one ($p < 0.01$), but higher during minute three ($p < 0.01$), and were equivalent across all other time-points (all p values > 0.05). During fixation, there was a main effect of *time*, $F(2,56) = 7.0$, $p < 0.01$, indicating blink counts increased overall with time. However, neither the main effect of *condition*, nor its interaction with *time* was significant (all F values < 1, all p values > 0.4). No other effects were observed in any of the other situations, (all F values < 2.28, all p values > 0.05). **Conclusions.** Being informed that ones blinks are being counted has a transitory impact on minute-to-minute blink counts, but not average blink rate while viewing images. **Acknowledgements.** This study was funded by the Neuroscience Research Unit of Pfizer Inc. The authors declare no competing interests.

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Poster

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Title: Frontal eye field correlates of saccade remapping and planning in natural scenes

Authors: *J. I. GLASER[1], P. RAMKUMAR[1], P. N. LAWLOR[1], D. K. WOOD[2], M. A. SEGRAVES[2], K. P. KORDING[1];
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Abstract: How is visual information transferred across eye movements (saccades) so that we can plan future saccades and maintain a stable perception of the world? One theory is remapping, where neurons' receptive fields (RFs) preemptively shift prior to saccade onset. According to this theory, neurons will respond to visual stimuli in their future receptive fields earlier than new visual information can reach the neurons. While remapping has been demonstrated in paradigms with flashed-on stimuli and uniform background images, it has not been investigated with stationary targets in complex natural scenes. Here, we analyzed recordings from the Frontal Eye Field (FEF), a region involved in saccade planning, while monkeys searched natural scenes for an embedded target. We found that during the saccade, or very soon after the saccade landing, many neurons had enhanced firing rates when the search target's final post-saccadic location would be in the neuron's receptive field (RF). We ensured that the response was in fact due to the target being in the upcoming RF rather than the previous RF (which often had significant overlap with the upcoming RF), using a generalized linear modeling approach. This enhanced response occurred too quickly for new visual information to have reached the FEF, suggesting that the enhanced response was in fact due to remapping. Moreover, we found that when the target was in the upcoming RF, a higher firing rate was predictive of the target being foveated more rapidly, thus demonstrating that this remapped visual information was used for saccade planning.

Disclosures: J.I. Glaser: None. **P. Ramkumar:** None. **P.N. Lawlor:** None. **D.K. Wood:** None. **M.A. Segraves:** None. **K.P. Kording:** None.

Poster

061. Eye Movements and Perception

Location: Hall A

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Topic: D.06. Eye Movements

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ERC Consolidator Grant 614244 P-CYCLES

Title: Brain circuits underlying visual stability across eye movements: oscillatory dynamics disentangle processing of multiple items

Authors: *D. MCLELLAND[1], J. SCHUSTER[2], F. HAMKER[2], R. VANRULLEN[1];
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Abstract: Despite our regular saccadic eye movements, our perceptual experience is of a stable visual world. This stability has been the focus of extensive research, and has been linked to saccadic remapping at the physiological level, and attention at the cognitive level. Ziesche and Hamker (2011, J Neurosci 31:17392; 2014, Front Comp Neuro 8:25) have developed a network model of the brain circuits underlying visual stability, and shown that it captures a set of important features characterizing peri-saccadic activity/behavior. Here, however, we show that testing with multiple simultaneous stimuli uncovers a shortcoming of the model: the stimuli are processed normally with independent "attention pointers" when they are spatially distant from each other, but interfere when close, such that they are represented by a single attention pointer midway between the two stimuli. We have recently shown that both attention and peri-saccadic remapping processes are linked to oscillatory brain activity at theta frequency (~7Hz). In the literature, one hypothesized role of oscillatory activity is in enabling the processing of multiple items by segregating the relevant neural activity in the temporal domain. We set out to test this theory in the context of the visual stability model. We first developed a spiking network version of the model (the original is rate coded), such that temporal interactions between units are meaningful at a timescale relevant for the oscillatory activity. Using this model, we were able to show how oscillatory neural activity can resolve the issue of item multiplexing. Specifically, we tested two major models of oscillatory processing: i) communication-through-coherence (Fries, 2009, Ann Rev Neurosci 32:209), by which coherent gamma frequency (~40 Hz) activity between brain regions enables selective processing of a single item, and a theta-frequency oscillation structures switching between items; and ii) theta-gamma coding (Lisman & Jensen 2013, Neuron 77:1002), whereby a different item is processed on each cycle of a gamma oscillation, and this ordered sequence of activity is repeated or updated on each theta cycle. Further, we explore the interaction between the timing of the neural oscillations and the saccade

event. These results serve both as a functionally useful and experimentally testable update to models of saccadic updating, and as a further example of how oscillatory processing may be a general mechanism across diverse brain operations.

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Poster

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Topic: D.06. Eye Movements

Support: DFG-SFB/TRR 135 / A1

Title: Directional precision for saccades and smooth pursuit in humans and macaque monkeys

Authors: *J. CHURAN[1], D. BRAUN[2], F. BREMMER[1], K. R. GEGENFURTNER[2];
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Abstract: Primates use a combination of saccades and smooth-pursuit eye movements (SPEM) to track moving targets. This requires a fast and accurate interaction of the two different oculomotor systems. We investigated the interaction of these systems by measuring the precision of eye movements in human subjects and in head-unrestrained non-human primates. We compared the directional precision of pure SPEM with the precision of a combination of an initial saccade followed by a SPEM. Combinations of initial saccades and SPEMs were elicited by the ramp paradigm while saccade-free SPEMs were produced in response to a step-ramp target with an appropriate timing. The motion direction was mainly horizontal with a randomly chosen vertical component in the range of +-20 degrees. We investigated how well the vertical component of the target motion was reflected in the eye-movement direction. To quantify the directional precision for the two conditions, oculometric functions were constructed. From these oculometric functions then direction thresholds were calculated for pursuit and saccades. These direction thresholds indicated the speed of vertical target motion required to influence the vertical eye-movement component. We found some differences in the absolute performance levels of humans and monkeys: Humans reached lower asymptotic thresholds than monkeys (1-2 deg for humans, ~6 deg for monkeys), while monkeys reached their asymptotic threshold much faster than humans (300-400 ms for humans, 200 ms for monkeys). Beyond these differences

humans and monkeys showed a remarkably similar pattern of differences between SPEMs and saccades. We found that, for both humans and monkeys in the ramp condition, the oculometric thresholds were significantly lower during the initial saccade than during pure pursuit responses at the same time relative to the onset of the stimulus. Thus we conclude that, while the saccadic system in humans and monkeys has a longer latency than the SPEM-system, it also has a better directional precision.

Disclosures: J. Churan: None. **D. Braun:** None. **F. Bremmer:** None. **K.R. Gegenfurtner:** None.

Poster

061. Eye Movements and Perception

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Support: NIH Grant 5R21DC013974-02

Ind State Dep Health A70-5-0791034

Title: Clinical utility of a rapid objective tool for diagnosing concussions via involuntary aspects of eye movements

Authors: *N. L. PORT, A. MADSEN, W. MEANS, T. LEELAND;
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Abstract: BACKGROUND: Critical decisions are made daily about whether to bench athletes who might have suffered mild traumatic brain injury (mTBI). Unfortunately, the low-level, diffuse damage underlying sport-related mTBI has proven difficult to measure, especially on the sidelines where initial decisions must be made quickly. Other than self-reported symptoms, the most widely used tool for diagnosing and tracking sports-related mTBI is the ImPACT test, a neuropsychological battery. This test is not viable as a rapid sidelines test because of its length (30 minutes) and the need for a controlled testing environment. It is also susceptible to motivational factors. The ideal instrument would be a) sensitive to low-level diffuse damage, b) easily and rapidly administered on the sidelines, and c) unaffected by human bias. Based on a body of research documenting the sensitivity of ocularmotor performance (e.g., eye movements such as saccades and smooth pursuit) to mTBI damage, we have built five Sideline Eye Trackers and are evaluating their clinical utility as a rapid, objective, and accurate sidelines test of sport-

related concussion/mTBI. METHODS: The Sideline Eye Tracker contains a 7" computer screen, works in direct sunlight, and is highly portable (e.g. fits in a backpack and is battery powered). Our current enrollment is over 1000 athletes, including the entire IU varsity athletic department, two high schools, a middle school and youth hockey and lacrosse teams. Subjects perform a six-minute ocular-motor exam consisting of two saccade tasks, two pursuit tasks, and an ocular following task. Balance data is collected simultaneously with a portable balance board. All athletes complete a baseline exam prior to the onset of their sport season. Anyone suspected of having a concussion is studied a minimum of 3 additional times: 1) immediately post-injury (minutes to within an hour), 2) at the time of being cleared for return-to-play, and 3) as far out post-injury as possible (~4-9 months). To provide two separate measures of test-retest variability, two control groups are also being studied, namely: 1) a within-sport and season-matched control group, and 2) non-concussions prone cross-country athletes. RESULTS: Preliminary results suggest that concussed athletes show significant deficits compared to their baseline measures in both saccades and pursuit. While data collection is ongoing, measuring ocular-motor deficits with our portable eye tracker and a 6 minute exam appears a promising method for diagnosing and tracking sport-related mTBI. Further data is needed in order to ascertain the specificity and sensitivity of our measures.

Disclosures: **N.L. Port:** None. **A. Madsen:** None. **W. Means:** None. **T. Leeland:** None.

Poster

061. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: NIH Grant MH104756

NIH Grant DC012087

Title: Natural viewing and pursuit behavior in marmosets performing foraging tasks

Authors: ***J. F. MITCHELL**[1], S. U. NUMMELA[2], C. T. MILLER[2];
[1]Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY; [2]Psychology, UCSD, La Jolla, CA

Abstract: Pursuit eye movements allow primates to stabilize high acuity central vision on slow moving targets. In natural conditions, this provides valuable visual information to guide

movements to those targets, for example to capture moving insects or to track conspecifics. In the laboratory, pursuit has been one of the best studied behaviors for understanding the feedback loop between sensory processing, target selection, and motor control. Recently we found that the small bodied New World primate, the common marmoset (Callithrix jacchus), shares core features of the pursuit behavior seen in humans and macaques (Mitchell, Priebe, and Miller, 2015). Because cortical areas crucial to pursuit (MT, MST, and FEF) are accessible at the surface of marmoset's smooth brains, and not buried in sulci, there are several potential advantages for recording and imaging in this species. However, the work ethic of marmosets is not the same as macaques, particularly for tasks where trials must be initiated from central fixation and where targets must be tracked over stereotyped trajectories to obtain reward. Here we instead examined pursuit behavior in the context of two foraging tasks that naturally engage marmosets to work for longer periods. In the first task, marmosets free viewed high resolution videos of other marmosets chasing each other in their home cages. We find that marmosets routinely pursue marmosets in these videos and that their pursuit shows visual selection of the target motion that is robust to competing background motion signals generated by eye movement. In a second task, marmosets actively tracked Gaussian windowed face images that moved along semi-random linear trajectories. In this task it is possible to manipulate the position and motion of appearing face targets based on the current eye position, providing greater control over sensory conditions. These foraging tasks will be of value for obtaining sufficient repetitions in marmosets, but more critically, may lead to a new understanding of how mechanisms of visual selection generalize to more dynamic natural viewing conditions.

Disclosures: **J.F. Mitchell:** None. **S.U. Nummela:** None. **C.T. Miller:** None.

Poster

061. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: Canada Foundation for Innovation

Title: Female sex steroid dynamics may modulate retinal-based horizontal smooth pursuit pathways

Authors: *E. CURRIE[1], M. F. WESNER[2];
[2]Psychology, [1]Lakehead Univ., Thunder Bay, ON, Canada

Abstract: Despite the existence of female sex-steroid receptors throughout the CNS including lower brainstem areas associated with oculomotor functioning, little is known about the non-reproductive effects of cycling sex steroids on CNS pathways. Given that there is converging evidence for depressive and cyclic hormonal influences on various eye movements in females, we decided to measure retinal-based smooth pursuit eye movements (SPEM) with the specific intent of localizing real-time tracking and tracking perseverance effects (i.e., extrapolated trajectories after target termination). Women underwent SPEM measurements during their late follicular (LF) and late luteal (LL) menstrual phases, where estradiol (progesterone) levels are known to be high (low) and low (high) respectively. To narrow down specific steroid effects, three groups of women were used who were (pro)retrospectively screened as either non-symptomatic, non-contraceptive users (Controls; N = 23), as having premenstrual syndromes (PMS; N = 22), or as using a monophasic contraceptive (Alesse™ users; N = 21). Based on salivary immunoenzymometric assay, our PMS and Alesse™ users showed stable estradiol levels across LL and LF phases with the latter also showing progesterone stability. Thus, certain group differences logically revealed estradiol- or progesterone-based manifestations on SPEM. Horizontal, sinusoidal tracking was done with a 60-Hz infrared eye tracker. Target excursion was ±13.3° from fixation moving at 0.25, 0.5 or 1.0 Hz. We found significant between-group deviations from nominal sinusoidal movements where PMS and Alesse™ users showed greater lag, amplitude excursions, and left-to-right excursion bias (greater average right than left amplitude). Our extrapolated measures showed shorter persistence for the PMS and Alesse™ users (defined as duration of movement that statistically followed an extrapolated track) with fitted decay functions revealing slopes that were steeper for the 0.5- and 1.0-Hz conditions compared to Controls. For the 1.0 Hz condition, Controls showed a periodic SPEM pattern that lasted until the end of a 2-sec recording. Also, there was an overall phase trend where LL showed steeper fitted decay slopes than LF. Because we found no direct menstrual phase effects outside of our assays, it is not clear whether estradiol or progesterone directly modulates SPEM circuits. However, based on our group differences, it is possible that neuroendocrine circuits in the CNS that invoke stable ovarian estradiol also modulate brainstem pathways that command SPEM maintenance such as those areas where estradiol has been shown to exert molecular effects.

Disclosures: **E. Currie:** None. **M.F. Wesner:** None.

Poster

061. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: UAB Department of Ophthalmology

Title: Conjugate eye movements to monocularly-visible and cyclopean targets

Authors: *J. S. DECKER, C. AURA, K. SCHULTZ, P. GAMLIN;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Saccades and smooth pursuit either shift or maintain gaze on objects of interest so that the image of the object is, respectively, either brought to or maintained on the fovea. Most previous studies have characterized these eye movements using monocularly-visible targets. However, eye movements can also be generated to stimuli that are purely cyclopean, i.e., visible only with binocular viewing and devoid of local contrast or other monocular cues. For future imaging or electrophysiological studies, we hypothesize that the use of cyclopean targets in addition to monocularly-visible targets will permit more effective dissection of the sensorimotor pathways involved in conjugate eye movement control. Therefore, the purpose of the present study was to test the feasibility of this approach by characterizing the metrics and dynamics of saccades and smooth pursuit to cyclopean targets and comparing these parameters to those for monocularly-visible targets. A custom-made stereoscopic visual display was used to present visual stimuli. Four participants performed horizontal saccades to targets at ±5° and ±10° and horizontal smooth pursuit eye movements over a range of 10° or 20° at 0.2 - 0.8Hz. Two types of monocularly-visible targets were used. One was a white cross subtending 1° on a dark background. The other was a 5° circular random dot stereogram (RDS) with +0.5° relative disparity with respect to a correlated dynamic RDS (DRDS) background. The cyclopean target was a 5° circular patch of DRDS with +0.5° relative disparity with respect to the DRDS background. This target was identical to the latter monocularly-visible target, apart from being redrawn in sync with the background redraw rate. Binocular eye positions were recorded using a 240 Hz eye tracking system (ISCAN). We found that saccadic gain was comparable for all target types; however, saccadic latency depended on target type. Shorter latencies were found for the non-RDS target; longer latencies were found for both the monocularly-visible RDS and cyclopean DRDS targets. For smooth pursuit at low frequencies, gain was near 1.0 and phase lag was minimal for all target types. At higher frequencies, gain was reduced for all target types, and phase lag was greater for the cyclopean targets. We conclude that humans are able to perform conjugate voluntary eye movements to cyclopean targets with dynamics and metrics qualitatively similar to those observed for movements to monocularly-visible targets. Therefore, cyclopean targets may be used in future experiments as a way to probe the neural circuitry underlying target selection and the sensorimotor transformations underlying eye movement control.

Disclosures: **J.S. Decker:** None. **C. Aura:** None. **K. Schultz:** None. **P. Gamlin:** None.

Poster

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Topic: D.06. Eye Movements

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CREAT371161-2009

Title: A computational model for feature integration across saccadic eye movements

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Abstract: Humans have a continuous visual perception of the world despite the fact that eyes collect visual information as discrete snapshot-like images across saccades. This raises several questions about human visual system. How does the brain make use of this temporally and spatially separated visual information to make a unified internal perception of the world? How does the visual system succeed in retaining, updating and integrating the visual feature information across saccades? The integration of visual information collected across saccadic eye movements is well known as trans-saccadic feature integration in the vision sciences literature. Here, we present a dynamic computational model to account for the mechanism of trans-saccadic feature integration across gaze shifts. The proposed model is a cyclic process consists of three phases: prediction, updating and fusion. We modeled short term memory as the stored internal representation of the most salient or task-relevant features in the visual scene. A prediction about the stored feature information at the time of the upcoming gaze position is then made based on efference copy signals (intended eye displacement, eye velocity, and eye position), so that the stored features are updated to be matched and consistent with the new gaze position. Afterwards, the spatially updated visual information is fed back to the fusion phase. The fusion phase also receives new visual feature information collected at the new gaze position and produces the desired fused (or integrated) feature information after the eye movement. Finally, the integrated output enters to the memory and updates the visual feature information stored in the memory. In the future, we are planning to make a neurally plausible version of this model to predict and interpret the neural mechanisms and underlying trans-saccadic integration. Supported by an NSERC Discovery Grant and the NSERC CANACT CREATE Program.

Disclosures: Y. Mohsenzadeh: None. **J. Crawford:** None.

Poster

061. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: P30EY003039

R01EY022290

R21EY018369

Title: Fmri targeted electrophysiology of a cyclopean stereomotion-in-depth region in rhesus monkeys

Authors: *K. SCHULTZ[1], M. WARD[2], M. BOLDING[2], P. GAMLIN[2];
[1]Univ. Alabama-Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: The perception of motion in depth in primates arises not only from the changing disparity of the images in the two eyes, but from a variety of monocular cues. To eliminate these cues, dynamic random dot stereogram (DRDS) stimuli can be used. These generate what appears to be random noise in either eye alone but, when binocularly fused, they generate the percept of a target with a given disparity. By systematically changing the disparity of the DRDS shown to the two eyes, one can produce the perception of cyclopean stereomotion in depth (csMID). In humans, a csMID region has been reported within hMT+ (Rokers et al., 2009) as well as immediately anterior to hMT+ (Likova and Tyler, 2007). To examine the neural processing underlying csMID perception in the rhesus monkey, we used fMRI to identify candidate regions. By interleaving csMID trials with trials using stimuli at fixed disparity planes, and by also comparing the results with the areas of activity related to eye movements in the same animal, we identified a region related to csMID in the superior temporal sulcus of two monkeys. Subsequently, recording chambers for electrophysiology were implanted and the position of these chambers was imaged using MRI, allowing us to target the csMID region for single-unit recording. Data (43 cells) from one animal has been collected to date. In order to differentiate cell activity for the fixed disparity plane versus motion-in-depth, we compared firing of the cells during near-to-far and far-to-near, as well as to different fixed disparity planes. Cell behavior was also characterized for conjugate smooth pursuit; saccades; dot field movements in multiple directions; dot fields shown to only one eye; uncorrelated dot fields; and dot fields that did not randomly refresh (monocular cues present). Consistent with the fMRI results, we encountered

some cells, generally deeper in the targeted area, that modulate their activity for csMID alone. In addition, the activity of other cells within the targeted area was modulated by vergence eye movements as well as by csMID. In the region superior to the csMID area, we encountered cells that modulate their activity in relation to vergence eye movements. Overall, we found that while cells in the targeted area code for cyclopean motion-in-depth, the activity of many are also modulated by disparity and eye movements. As expected from the overlapping areas of activity for eye movements and csMID in fMRI, the cells found in and around the targeted area have complex visual and oculomotor properties that warrant further investigation.

Disclosures: K. Schultz: None. **M. Ward:** None. **M. Bolding:** None. **P. Gamlin:** None.

Poster

062. Persistent Pain Treatment

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 62.01/L9

Topic: D.08. Pain

Title: External directed high intensity focused ultrasound for migraine treatment in rodents

Authors: *J. G. PILITSIS[1], L. GEE[2], G. GHOSHAL[3], I. WALLING[4], C. E. BURDETTE[3]; [1]Neurosurg., [2]Ctr. for Neurosci. and Neuropharm., Albany Med. Col., Albany, NY; [3]Acoustic MedSystems Inc, Savoy, IL; [4]Dept. of Neurosurg., Albany Med. Ctr., Albany, NY

Abstract: Chronic migraine affects over 37 million people in the US and up to 15% are refractory to medical treatment. Interestingly, 70% of patients exhibit allodynia, an increased sensitivity to non-painful stimuli, which we can study using a validated rodent model of chronic migraines (CM) which also causes allodynia in the peri-orbital region. We investigate a novel external high intensity soft focused ultrasound system (HI-SFUS) on allodynia in a rodent model of headache. Using epidurally infused inflammatory media for 15 applications, we induce a chronic migraine state in Sprague Dawley rats. We use a unique HI-SFUS external applicator to deliver 3-6 watts of acoustic energy to the occipital region of the rats, an area that is frequently blocked or stimulated in patients for temporary control of migraines (Fig 1). Our device allows directed high frequency ultrasound ablative energy to be directed specifically at the occipital nerves non-invasively setting it apart from other therapies. Mechanical thresholds of the peri orbital region were assessed using vonFrey filaments. We found a significant reduction in mechanical thresholds after inducing the CM state in rodents (n=4, p=0.02). Immediately after HI-SFUS, thresholds were significantly increased and remained increased significantly at 1 day post treatment (Fig 2, p=0.048). Thresholds remained elevated for the first 6 days following

ultrasound therapy. We demonstrate proof of principle of externally applied high intensity soft focused ultrasound therapy in a rodent migraine and demonstrate significant improvement of allodynia following HI-SFUS administration. We are currently performing a dose response study to assess the optimal, safe dose of energy to administer to improve allodynia. Further device customization will allow preferential targeting of the nerve at the exact depth and location to illicit optimum response.

Figure 1. External HIFU applicator device

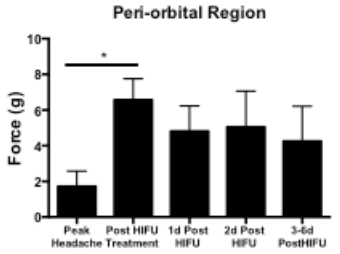

Figure 2. Mechanical thresholds in peri-orbital region. Thresholds increased significantly after HIFU treatment (p=0.048) and remained elevated up to 6 days afterwards

Disclosures: **J.G. Pilitsis:** 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 1R01CA166379. F. Consulting Fees (e.g., advisory boards); Medtronic, St. Jude, Boston Scientific. **L. Gee:** None. **G. Ghoshal:** A. Employment/Salary (full or part-time):; Acoustic MedSystems Inc. **I. Walling:** None. **C.E. Burdette:** A. Employment/Salary (full or part-time):; Acoustic MedSystems Inc.

Poster

062. Persistent Pain Treatment

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Topic: D.08. Pain

Support: DA018156

UNM Anesthesia Research Fund

Title: Chronic peripheral nerve injury produces phase-selective suppression of voluntary wheel activity in rats consistent with the duration of bilateral sensory allodynia

Authors: *R. WHITEHEAD[1], M. S. SUN[2], J. J. SANCHEZ[2], H. MARTIN[2], E. MILLIGAN[2]; [1]Univ. of New Mexico, Santa Fe, NM; [2]Univ. of New Mexico, Albuquerque, NM

Abstract: Peripheral neuropathies such as heightened sensitivity to light touch (clinically referred to as allodynia) are an underlying cause of many types of chronic pain arising from aberrant glial and neuronal signaling. One method commonly used to detect allodynia in rodent models of peripheral neuropathy is the von Frey test (VF), which utilizes a series of calibrated monofilaments applied to the hindpaw eliciting a paw withdrawal response. One widely applied rodent model of peripheral neuropathy is unilateral sciatic nerve chronic constriction injury (CCI) that generates reliable allodynia. Complementary non-reflexive physical-activity measures such as voluntary wheel running (VWR) may be useful to assess function, as reduced activity is a common feature in clinical neuropathic pain. Here, we aimed to determine whether VWR would reveal a reduction in physical activity (distance traveled) under peripheral neuropathic conditions. Three experimental groups were assigned to running wheels (RW) at the onset of the rat's light cycle: (1) CCI with unrestricted access to in-cage RWs, (2) sham surgery with unrestricted access to in-cage RWs, and (3) CCI with unrestricted access to locked in-cage RWs. An additional group was run to determine the effects of CCI on VWR at the onset of the rat's dark cycle. Seven & 3 days prior to surgery, RW activity levels and baseline (BL) paw withdrawal thresholds (VF test), respectively, were obtained. Daily RW activity levels (1 hr) were obtained each 24 hr for 7 days prior to surgery. After surgery, hindpaw thresholds followed by RW activity (1 hr) were re-assessed 3, 10, 14, and 18 days after surgery, with an extra VF timepoint collected on Day 16. RWs were connected to an automated data system that recorded the absolute values of distance traveled (meters). Rats were monitored continuously during the 1-hr RW exposure. Data were acquired every second allowing for analyses of binned 20-min profiles: Phase I, II and III within the 1 hr period. We observed similar allodynia profiles between wheel-locked and RW groups, suggesting that 1 hr WR exposure does not alter the VF thresholds during peripheral neuropathy. Compared to sham controls, robust decreases in RW activity was observed on days 3, 10 & 14 post-surgery in CCI rats, while allodynia remained significant at all timepoints. The most pronounced decrease in RW activity was observed during Phase II, with similar decreases observed in rats expose to WR during the dark cycle, with about 50% greater overall activity levels than light-cycle activity levels. VWR may serve as a complementary assay of peripheral neuropathy in rats, which could be useful in providing a screen for novel pain therapeutic drugs.

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Poster

062. Persistent Pain Treatment

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Support: Grants from Platform for Drug Discovery, Informatics, and Structural Life Science from MEXT.

Title: Donepezil completely reverses fibromyalgia-like pain in intermittent cold stress model mice

Authors: *H. NEYAMA, T. MUKAE, H. UEDA;
Nagasaki Univ., Nagasaki-Shi, Japan

Abstract: We have started to search for therapeutic candidates to cure experimental fibromyalgia-like pain disease. Our strategy is to use intermittent cold stress model to cause fibromyalgia-like pain in mice. The model mouse shows chronic pain (thermal, mechanical, chemical and electrical) for over 3 weeks, female-predominant gender difference, lack of morphine analgesia, but sensitive to pregabalin (i.c.v.) and antidepressants (i.t.). However, constant cold stress showed only a transient pain, which completely disappears 5 days after the stress. Based on clinical evidence that fibromyalgia often accompanies the symptoms, dry-eye and dry-mouth, we started pilocarpine to treat this chronic pain disease. Following successful results and identification of brain-specificity, we further observed that efficiently brain-penetrable donepezil could completely cure this pain disease even after repeated treatments. In the present study we will discuss the newly produced pain-inhibitory mechanisms following repeated donepezil treatments in terms of brain loci and related genes.

Disclosures: **H. Neyama:** A. Employment/Salary (full or part-time):; Graduate Student, Pharmacology and Therapeutic Innovation,Nagasaki University,Nagasaki,Japan. **T. Mukae:** A. Employment/Salary (full or part-time):; PhD Student. **H. Ueda:** A. Employment/Salary (full or part-time):; Professor, Pharmacology and Therapeutic Innovation,Nagasaki University,Nagasaki,Japan.

Poster

062. Persistent Pain Treatment

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 62.04/L12

Topic: D.08. Pain

Support: AHW Endowment Project 5520293

Title: AAV6 based delivery of zeta inhibitory peptide to dorsal root ganglion neurons and its effect on nerve-injury induced neuropathic pain

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Abstract: Protein Kinase C-zeta and Protein Kinase M-zeta (PKCζ and PKMζ) are atypical PKC (aPKC) isoforms with demonstrated roles in altering synaptic strength in learning and memory. Evidence also exists that aPKCs have similar effects on nociceptive pathways in chronic pain states. Bulk administration of the selective PKC/Mζ pseudosubstrate inhibitor zeta inhibitory peptide (ZIP) to multiple sites in the central nervous system has shown efficacy in preventing or reversing increased sensitivity after peripheral inflammation or nerve injury. PKMζ is highly enriched in sensory neurons in the dorsal root ganglion (DRG), but the role of aPKCs in peripheral sensory neurons remains relatively unexplored. We determined whether inhibition of PKC/Mζ activity in primary sensory neurons had an effect on ongoing neuropathic pain. Using an adenoassociated virus (AAV 2/6) encoding ZIP linked to GFP (AAV6 GFP-ZIP) injected into fourth and fifth lumbar DRG, we examined whether inhibition of PKC/Mζ affected the evolution of neuropathic pain after tibial nerve injury (TNI) compared to controls. Tissue was histologically examined to characterize sensory neuron transduction, and to determine if aPKC inhibition had effects on phenotypic changes in DRG neurons typical of nerve injury. Vector injection into L4 and L5 DRGs resulted in efficient and persistent transduction of a wide phenotypic range of sensory neurons, and revealed transgene expression that extended to the central terminals in the spinal cord dorsal horn. Although nerve injury did not alter total expression of PKC/Mζ in DRG neurons, enzyme inhibition reduced the expression of pain-related behaviors four weeks after injury. This could reflect an effect on changes in synaptic transmission that endure after the response to the initial insult had subsided. These findings suggest that PKC/Mζ are an important component of processes governing the response of neurons to stimulation, and that aPKCs may present a potential therapeutic target for modifying the evolution of chronic pain and other disorders of neuronal and synaptic function.

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Poster

062. Persistent Pain Treatment

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Topic: D.08. Pain

Support: Taiwan National Science Council Grant NSC 103-2321-B-214-001-

E-Da Hospital Grant EDPJ 102043, 102044, 103054, 103055 Taiwan

Title: The antinociceptive effect of light emitting diode irradiation on incised wound is via inhibition of cyclooxygenase-2 and prostaglandin E2

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Abstract: Introduction: Light emitting diode (LED) phototherapy has attracted attention for reducing pain and inducing tissue repair through several mechanisms. Optimal postsurgical pain therapy remains a challenge for physicians. The analgesic effect of LED on incised wound has never been examined. In this study, we examined the analgesic effect on incised pain and the change of cyclooxygenase 2 (COX-2) and prostaglandin E2 (PGE2) after LED therapy. Methods: The animal protocols were approved by the Institutional Review Board of I-Shou University, Kaohsiung, Taiwan. The rats were randomly assigned to the following groups. Rats received LED therapy on incised skin 6 days before incision (LI group) or 6 days after incision (IL group) or from 3 days before incision to 3 days after incision (LIL group) and skin incision only (I group). Thermal hyperalgesia and mechanical allodynia were tested 1 day after incision in LI and I groups or after LED therapy in the other groups; skin tissues were collected for RNA and protein analysisof COX-2 and PGE2 (n = 6 each group) after behavior test. The RNA and protein analysis are performed by real time quantitative PCR and western blot. Results: Significant thermal hyperalgesia (lower thermal withdrawal latency) was noted in I group compared with the other three LED-treated groups. The expression of COX-2 and PGE2were significantly decreased in the three LED-treated groups compared with I group. Concludions: We concluded LED therapy could relieve thermal hyperalgesia on incised wound and the analgesic effect is possibly produced by inhibiting the expression of COX-2 and PGE2.

Disclosures: P. Tan: None. **C. Liu:** None.

Poster

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Title: Intrathecal bone marrow stromal cells inhibit neuropathic pain via TGF-β secretion and target dorsal root ganglia via CXCL12

Authors: *G. CHEN, C.-K. PARK, R.-G. XIE, R.-R. JI;
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Abstract: Neuropathic pain remains a pressing clinical problem and there are still no drugs that can treat neuropathic pain in a complete and definitive way. Bone marrow stromal cells (BMSCs) are a population of progenitor cells of mesodermal origin and present in the bone marrow of adults, emerging as a major source for cell-based therapies for clinical applications. BMSCs were originally conceived as stem/progenitor cells to rebuild diseased or damaged tissues. However, systemically infused BMSCs have been shown to exert therapeutic effects through the release of cytokines/trophic factors that act on local, or perhaps distant, target tissues. Through this paracrine modulation, it has been discovered that BMSCs are potent modulators of immune responses in humans and animals. Here we report that a local injection of BMSCs, via intrathecal route following lumbar puncture, can prevent and reverse neuropathic pain symptoms (allodynia and hyperalgesia) in mice for several weeks following nerve injury (chronic constriction injury, CCI). Intratehcal BMSCs also reduced CCI-induced ongoing pain as measured by conditioned place preference. Furthermore, this BMSCs treatment protected dorsal root ganglion (DRG) neurons from nerve injury and inhibited neuroinflammation in DRGs and spinal cords. Interestingly, BMSCs secreted TGF-β1 to CSF, and the analgesic effect of BMSCs was reversed by neutralization of TGF-β1 but not IL-10. Conversely, intrathecal administration of TGF-β1 potently inhibited neuropathic pain. TGF-β1 is a powerful neuromodulator and rapidly (within minutes) suppressed CCI-evoked spinal synaptic plasticity and DRG neuronal hyperexcitability via TGF-β receptor-1-mediated non-canonical signaling. CCI also upregulated CXCL12 in lumbar L4-L6 DRGs, and this up-regulation caused migration of intrathecally injected BMSCs to L4-L6 DRGs through CXCR4 expressed on BMSCs. These migrated BMSCs

survived in DRGs for more than two months and eventually disappeared. Finally, intrathecal BMSCs also effectively reduced neuropathic pain after spared nerve injury. Our findings support a paracrine mechanism by which intrathecal BMSCs target the CXCL12-producing DRGs via to elicit neuroprotection and sustained neuropathic pain relief via TGF-β1 secretion. Key words: bone marrow stromal cells, intrathecal injection, TGF-β1, CXCL12, CXCR4, neuropathic pain

Disclosures: G. Chen: None. **C. Park:** None. **R. Xie:** None. **R. Ji:** None.

Poster

062. Persistent Pain Treatment

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Topic: D.08. Pain

Support: CGH-MR-A10307

Title: Co-adminstration of melatonin attenuates morphine tolerance and preserves morphine's antinociceptive effect in spinal nerve ligation rats

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Abstract: Opioids possess strong antinociceptive effect on various types of pain in clinical; however certain types of pain are difficult to control with opioids. For example, neuropathic pain, caused by nerve injury, is poorly responded to morphine at regular dose. Recently, pineal hormone melatonin was demonstrated to exert antinociceptive effect in animal models, including neuropathic pain models. However, the effects of chronic melatonin treatment and the combination effect of melatonin with morphine are still unclear. In the present study, we explore the combined effect of melatonin with morphine on neuropathic pain and the underlying mechanisms. Spinal nerve ligation or sham operation was performed in adult male Wistar rats. Melatonin (10 mg/kg, i.p.) was co-administrated with morphine (5 mg/kg, s.c.) 1 day after surgery for 14 days. Anti-allodyniic and anti-hyperalgesic effects were assessed by von Frey test and plantar test before and after drug treatment. The L5 spinal cord was removed for western blot, immunohistochemistry or cytokine assay after all the behavioral tests. Our data show that chronic morphine treatment induced tolerance not only in rats underwent sham operation but also in rats with neuropathic pain. Co-administration of melatonin prevents morphine-induced tolerance and increase morphine's antinociceptive effect in spinal nerve ligation rats. We

conclude that co-administration of melatonin with morphine could be a future therapeutic paradigm for the management of chronic neuropathic pain.

Disclosures: **J. Kao:** None. **C. Wong:** None.

Poster

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Support: No. 13430722100 From The Science and Technology Commission of Shanghai Municipality, Shanghai, China

No. XBR2011024 From The Shanghai Bureau of Health, Shanghai, China

Title: Analgesic and neuro-protective effect of acrolein-scavenger of phenelzine for rats with spinal cord injury

Authors: ***Z. CHEN**[1], J. PARK[2], G. GLEN[2], R. TIAN[2], S. VEGA-ALVAREZ[2], P. CAO[3], R. SHI[2];
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Abstract: Acrolein, an aldehyde and endogenous byproduct of lipid peroxidation, is reported to play an important role in neuropathic pain (NP) and neuro-tissue damage after spinal cord injury (SCI). In addition, hydralazine, a known acrolein scavenger, has been shown to suppress acrolein and mitigate sensory hypersensitivity post-SCI. We have found that systematic administration of phenelzine, another known acrolein-scavenger with a chemical structure distinct of that of hydralazine, also has the ability to alleviate NP when administered either immediately post injury, with a delay of three weeks, or a further delay of two months post SCI. Consistent with its neuroprotective effect, the application of phenelzine was also effective in ameliorating motor deficits and preserving neuro-tissues after SCI. In fact, phenezline produced a higher efficacy of analgesic effects compared to hydaralzine when both were used at a maximal safe level. We also show that acrolein-adducts were reduced due to phenelzine application in spinal cord tissue based on immunoblotting techniques. This is in good agreement with the reduction of 3-

hydroxypropyl mercapturic acid (3-HPMA), a metabolite of acrolein in urine, following the application of phenelzine. Furthermore, phenelzine application has also resulted in the reduction of post-SCI elevation of TRPA1 mRNA in central and peripheral locations. Taken together, we have shown that phenelzine is capable of mitigating NP by neutralizing excessive acrolein accumulated post-SCI and also reducing the expression of TRPA1 mRNA. Considering its longer half-life *in vivo* (11 hrs) compared to hydralazine (1 hr), and higher efficacy in mitigating pain, phenelzine is likely a viable, alternative acrolein scavenger in reference to hydralazine that can provide neuroprotection and analgesic effects post SCI.

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Poster

062. Persistent Pain Treatment

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 62.09/L17

Topic: D.08. Pain

Title: Virtual reality and modulation of embodiment for controlling chronic pain

Authors: *M. SOLCÀ[1,2], R. RONCHI[1,2], J. BELLO RUIZ[1,2], T. SCHMIDLIN[2], A. SERINO[1,2], B. HERBELIN[1,2], F. LUTHI[3], J.-Y. BEAULIEU[4], A. SCHNIDER[5], A. GUGGISBERG[5], O. BLANKE[1,2,6];
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Abstract: Introduction: Complex regional pain syndrome (CRPS) is a chronic painful condition that remains poorly understood and difficult to treat. Recent work has applied insights from the neuroscience of embodiment and body ownership to moduale pain perception and showed that inducing body ownership for artificial body parts results in analgesia for experimentally-induced pain in healthy subjects. Here we adapted the existing research protocole and translated it to the study of chronic pain in patients suffering from CRPS. Methods: 14 patients with CRPS following upper limb trauma were tested. We used physiologically-enhanced virtual reality and exposed patients to cardio-visual stimulation, in which we presented participants with a virtual

hand flashing in synchrony (or in asynchrony in the control condition) with respect to their own online-detected heartbeats. Prior and after each experimental condition, we assessed pain, grip strength, and embodiment measures. Results: Preliminary results demonstrated that the large majority of CRPS patients well supported the entire procedure and embodied the virtual hand, independently of stimulation condition. However, reduction of pain ratings was stronger in the synchronous as compared to the control (asynchronous) condition. Importantly, grip strength increased after exposure to the synchronous condition probably through the embodiment analgesic effect. Conclusion: This study describes a novel approach to achieve pain relief in patients with chronic pain through the use of physiologically-enhanced virtual reality. These preliminary data show that exposure to specific patterns of cardio-visual illumination of a virtual hand, with related effects of embodiment, can be used to treat CRPS symptoms, potentially leading to new non-invasive, analgesic rehabilitation programs for different pain conditions.

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Poster

062. Persistent Pain Treatment

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Topic: D.08. Pain

Title: miRNA-132-3p plays a role in chronic neuropathic pain

Authors: *M. LEINDERS[1], N. ÜÇEYLER[1], C. SOMMER[1], L. SORKIN[2];
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Abstract: Chronic pain is a frequent and serious condition that reduces quality of life and is often resistant to treatment. MicroRNAs (miRs) are master-regulators of multiple intracellular pathways that are implicated in the pathophysiology of pain. In particular, miR-132 seems to be an interesting candidate, based on preliminary findings in patients with painful polyneuropathies. We therefore assessed miR-132 expression in an animal model of neuropathic pain, and furthermore, studied its functional relevance in spontaneous and evoked pain behavior, including the place escape avoidance paradigm (PEAP). We systematically assessed miR-132-3p and miR-132-5p isoform expression throughout the neuraxis of male Sprague-Dawley rats before and after spared nerve-injury (SNI) in comparison to sham-operated littermates. An intrathecal (i.th.)

LNA-miR-132-3p inhibitor was used to assess the functional role of miR-132, 10 days after SNI. Tests for pain and avoidance behavior were performed prior to, and after SNI and/or followed by i.th. injections by assessing paw withdrawal thresholds with von Frey filaments and with the PEAP. MiR-132-3p expression was higher in the ipsi- but not contralateral L4/5 spinal cord and dorsal root ganglia (DRG) of SNI rats showing pain behavior compared to samples from sham controls (2.4 fold change; $p<0.05$ for both). In contrast, miR-132-5p did not show any difference in expression. I.th. administration of the miR-132-3p antagonist reduced SNI-induced spinal and DRG expression of miR-132 (10.8 fold change $p<0.01$), whereas scr-mismatch control had no effect. Animals that received anti-miR-132-3p showed dose-dependent reduction of mechanical hyperalgesia lasting for up to 7 days after the last (3rd) daily bolus injection ($p<0.001$), compared to animals treated with scr-mismatch control. In the PEAP test, SNI animals spent significantly more time in the light chamber when time in the dark chamber was matched with stimulation of their SNI paw, however animals given the miR-132 inhibitor spent significantly more time in the dark chamber under the same experimental conditions ($p<0.001$), indicating that they no longer found the dark chamber aversive. We found evidence for elevated spinal and DRG miRNA-132-3p expression and its involvement in pain behavior. We identified miRNA-132-3p but not -5p as being involved in not only evoked pain but also pain-aversion. Further studies will unravel downstream mechanisms and pathophysiological significance of miR-132 in chronic pain.

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Poster

062. Persistent Pain Treatment

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Title: Carbamazepine potentiates the effectiveness of morphine in a rodent model of neuropathic pain

Authors: A. L. RANDOLPH[1,3], M. S. RIPSCH[1,3], Y. M. ALLETTE[1], M. R. DUE[1], Y. KIM[1], C. ALBANY[2], *F. A. WHITE[4,3];
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Abstract: Approximately 60% of morphine is glucuronidated to morphine-3-glucuronide (M3G) which may aggravate preexisting pain conditions. Accumulating evidence indicates that M3G signaling through neuronal Toll-like receptor 4 (TLR4) may be central to this proalgesic signaling event. These events are known to include elevated neuronal excitability, increased voltage-gated sodium (NaV) current, tactile allodynia and decreased opioid analgesic efficacy. Using an *in vitro* ratiometric-based calcium influx analysis of acutely dissociated small and medium-diameter neurons derived from lumbar dorsal root ganglion (DRG), we observed that M3G-sensitive neurons responded to lipopolysaccharide (LPS) and over 35% of these M3G/LPS-responsive cells exhibited sensitivity to capsaicin. In addition, M3G-exposed sensory neurons significantly increased excitatory activity and potentiated NaV current as measured by current and voltage clamp, when compared to baseline level measurements. Using a known inhibitor of several NaV currents, carbamazepine (CBZ), we then compared the efficacy of CBZ and morphine as independent agents to the combined treatment of both drugs simultaneously in a variant of the spared nerve injury model of neuropathic pain; tibial nerve injury (TNI). The potent anti-nociceptive effects of morphine (5 mg/kg, i.p.) were observed in TNI rodents at post-injury day (PID) 7-14 and absent at PID21-28, while administration of CBZ (10 mg/kg, i.p.) alone failed to produce anti-nociceptive effects at any time following TNI (PID 7-28). In contrast to either drug alone at PID28, the combination of morphine (1 or 5 mg/kg, i.p.) and CBZ transiently reversed tactile hyperalgesia in the rodent TNI model. Taken together, our observations demonstrate a potential therapeutic use of morphine and CBZ as a combinational treatment for neuropathic pain conditions. An open-label, single arm, Phase Ib dose escalation study of oxcarbazepine (structural derivative of carbamazepine)with morphine in patients with chronic pain is currently evaluating the safety and toxicity of the combination of these two FDA-approved drugs (https://clinicaltrials.gov/ct2/show/NCT02078089). The secondary endpoints are improving pain control, reduce morphine use and improve the quality of life.

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Poster

062. Persistent Pain Treatment

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Topic: D.08. Pain

Support: Palmer Center for Chiropractic Research

Title: Spinal manual therapy effects on experimental pain

Authors: *S. M. ONIFER[1], W. R. REED[1], R. S. SOZIO[2], C. R. LONG[3];
[1]Associate Professor, [3]Professor, [2]Palmer Ctr. For Chiropractic Res., Davenport, IA

Abstract: Neuromusculoskeletal pain is often managed clinically by non-pharmacologic complementary and integrative health manual therapies. Optimizing pain relief resulting from manual therapies requires studies that determine their biologic effects and mechanisms of action. Essential to these studies are experimental pain models wherein simulated manual therapies are administered and their effects on nociceptive events are assessed by established methods. Short-term, remote pain relief occurs after a single spinal manual therapy treatment. We simulated a spinal manual therapy passive lumbar flexion technique in adult male Sprague Dawley rats and investigated the duration of anti-nociceptive effects following a single treatment on nociceptive behavior during the hindpaw formalin test. Nociceptive events in this model of peripheral pain occur during a rapid-onset, brief acute phase and a persistent phase separated by a quiescent interphase. Dilute formalin was injected subcutaneously at the plantar surface of the hindpaw. Nociceptive behavior during the acute phase was video-recorded for 5 minutes. Ten minutes of cyclic 20º passive flexion was administered with a custom-made device at the lumbar (L5) vertebra of isoflurane-anesthetized experimental rats (n=12) beginning 10 minutes after formalin injection. Nociceptive behavior during the persistent phase was video-recorded for 60 minutes beginning 5 minutes after treatment ended. Control rats (n=12) underwent the same methods except the passive lumbar flexion. The mean times spent licking the formalin-injected hindpaw of both groups during 1-5 minutes following formalin injection, and prior to passive lumbar flexion, were not different. The mean licking times of experimental rats during the first 20 minutes after passive lumbar flexion were significantly less than that of control rats ($p<0.001$). The mean licking times of both groups during the second and third 20 minutes following passive lumbar flexion were not different. These findings are similar to the short-term, remote pain relief reported clinically after a single spinal manual therapy treatment. Thus, this simulated manual therapy technique and experimental pain model can be used for investigations of peripheral and central mechanisms of action that are needed to optimize the clinical efficacy of non-pharmacologic complementary and integrative health interventions.

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Poster

062. Persistent Pain Treatment

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Topic: D.08. Pain

Support: Spinal Moduation Inc. Research Support

Title: Field stimulation of the dorsal root ganglion reduced noxious stimulation-induced cortical activation

Authors: C. PAWELA, Z. LI, A. KACZMAROWSKI, *Q. H. HOGAN;
Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Spinal cord stimulation is a well-established treatment option for chronic pain that electrically activates predominantly Abeta sensory neuron fibers in the dorsal columns. An alternative pain treatment approach is being developed that uses electrical field stimulation of the dorsal root ganglion (ganglionic field stimulation - GFS), where nociceptive neurons may also be activated. This has shown initial success in clinical trials. We have developed a rat model of GFS, and here test its efficacy against acute noxious stimulation using function magnetic resonance imaging (fMRI) imaging to gauge it effect on pain-related activation of brain regions. Male rats (160-260g) were anesthetized with dexmedetomidine (0.05mg/kg/hr) and pancuronium (2mg/kg/hr), mechanically ventilated, and subjected to noxious electrical stimulation of the hind paw (3ms pulses at 10Hz, 5mA), which activated brain centers including hindlimb primary somatosensory cortex (S1HL), anterior cingulate cortex, insular cortex, thalamus, and caudate/putamen. Subsequent presentation of the same noxious stimulation during GFS (20Hz, 0.3ms, current at a level determined to be subthreshold for motor activation; n=9) delivered by a bipolar electrode (1mm separation) previously placed into the intervertebral foramen such that it was adjacent to the L4 DRG, showed reduced activation of S1HL, thalamic ventral posterolateral nucleus (VPL), and anterior cingulate. In contrast, a repeated presentation of the noxious stimulation without GFS (n=4) showed no changes from the initial noxious stimulation. GFS alone (without noxious paw stimulation; n=11) showed no discernable fMRI activation when delivered at the therapeutic level, but increasing GFS intensity (5mA) resulted in patterns comparable to noxious paw stimulation - which together show that the treatment level was sub-nociceptive. We conclude that GFS is antinociceptive, but does not produce a simple afferent blockade. Rather, GFS selectively inhibits activation of S1HL and VPL (sensory-discriminative function) and anterior cingulate (affective-motivational), while other centers activated by noxious stimulation are not affected.

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Poster

062. Persistent Pain Treatment

Location: Hall A

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Program#/Poster#: 62.14/L22

Topic: D.08. Pain

Title: Creation of a brain-penetrant peptide-neurotensin(8-13) conjugate exerting analgesic activities after systemic administration

Authors: ***J. COTE**[1], M. DEMEULE[2], N. BEAUDET[1], A. REGINA[2], K. BELLEVILLE[1], A. LAROCQUE[2], J.-M. LONGPRÉ[1], J. LACHOWICZ[2], J.-P. CASTAIGNE[2], P. SARRET[1]; [1]Pharmacol. and Physiol., Univ. De Sherbrooke (FMSS), Sherbrooke, QC, Canada; [2]Angiochem Inc., Montreal, QC, Canada

Abstract: Neuropeptides play a crucial role in brain functions, and peptide receptors hold great promise in advancing structure-based drug discovery for the treatment of brain disorders. However, one of the major remaining challenges in the development of peptides as potential drugs is to achieve therapeutic brain concentrations after systemic delivery, the blood-brain barrier (BBB) preventing entry of molecules from cerebral capillaries into surrounding brain tissue. In the present study, we conjugated the biologically active neurotensin fragment (NT(8-13)), which produces strong analgesia when injected directly into the brain, to the Angiopep-2 peptide (An2), a proprietary 19-amino acid peptide that crosses the BBB by LRP1 receptor-mediated transcytosis. The brain uptake of this new chemical entity, An2-NT(8-13), was first determined using positron emission tomography coupled to computed tomography (PET/CT) imaging. For this purpose, we acquired dynamic PET scans over 60 min followed by a CT scan and quantified brain distribution of 64Cu-radiolabeled An2-NT(8-13) with or without pre-blockage of LRP1 receptors with an excess of unlabeled An2. These experiments showed that An2-NT(8-13) accumulates more efficiently in the brain when pre-blockage is not performed, thus demonstrating transcytosis through a LRP1-dependent mechanism. We next investigated whether the An2-NT(8-13) conjugate exhibited potent analgesic activity in different pain models. In rats, An2-NT(8-13) administered intravenously (i.v.) attenuates the stereotypical nociceptive

behaviors observed following intraplantar injection of formalin into the right hind paw (formalin tonic pain model). At a dose of 0.05 mg/kg, An2-NT(8-13) was also effective in reversing the pain behaviors induced by chronic constriction injury of the sciatic nerve (neuropathic pain). Finally, we found that i.v. An2-NT(8-13) significantly reversed the allodynic state induced by the femoral inoculation of MRMT-1 rat breast cancer cells (bone cancer pain). Altogether, these results demonstrate that the An2-NT(8-13) derivative penetrates the BBB efficiently after systemic administration and mediates relief of chronic pain, thus supporting the potential of An2-NT(8-13) as a first-in-class NT-based chronic pain therapeutic.

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Poster

062. Persistent Pain Treatment

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 62.15/L23

Topic: D.08. Pain

Support: NIH Grant DK105687

Integrated Tissue Dynamics LLC

Title: Depletion of heat sensitive TRPV1 positive cutaneous nociceptors by infrared light

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Abstract: Painful peripheral neuropathy (PPN) can have a variety of causes, including diabetes, HIV, herpes zoster, and chemotherapy. PPN poses a considerable challenge to physicians because it is difficult to diagnose and current treatments are usually inadequate with fewer than one third of patients achieving no more 50% pain relief. Most therapeutics target purported pain mechanisms in CNS producing unwanted side effects. Some alternative strategies attempt to reduce or eliminate demonstrated pathological hypersensitivity of cutaneous sensory endings of peripheral nociceptive neurons. Capsaicin is a TRPV1 agonist which, when applied to the skin, has been shown to deplete sensory endings in the epidermis that are postulated to express TRPV1 and one of the main sources of neuropathic pain. Unfortunately, the half-life of capsaicin in the skin is ~ 24 hours and can therefore lead to prolonged inflammatory pain after treatment. This post treatment pain together with non-specific chemotoxicity limit the use of high concentration capsaicin patches even for FDA approved treatment of some types of PPN. However, heat also acts as physical agonist of TRPV1 channels. Subsequently, we have shown that diode laser (DL) pulses can selectively activate TRPV1 positive neurons *in vitro* and *in vivo*. In this study we provide preliminary evidence from punch biopsies of pig skin, that 4 Watt DL pulses deplete sensory endings immunoreactive for PGP9.5, a widely (and clinically) used cross-species marker of cutaneous innervation. As much as 80% of the sensory innervation to the epidermis has been shown to co-express TRPV1 immunoreactivity in healthy subjects and patients with DPN. Moreover, TRPV1 is also expressed in keratinocytes whose stimulation may in turn activate epidermal endings that lack direct expression of TRPV1. The punch biopsies were taken at seven days after repetitive DL pulses and show that this depletion is dose-dependent in that 10 pulses did not reduce innervation density over control tissue but 25 pulses produces a substantial decrease. Importantly, the higher dose caused no obvious damage or inflammation (e.g., macrophages) as assessed sections stained for hematoxylin and eosin (H&E), a standard histological preparation for examining damage and inflammation of skin.

Disclosures: M.I. Nemenov: None. **F.L. Rice:** None.

Poster

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Topic: D.08. Pain

Support: 1ZIAAT00002302

Title: Amount of exercise is not related to exercise-induced analgesia in a rat model of persistent inflammatory pain

Authors: *F. TARUM[1], M. PITHCER[2], R. IMRAN[2], M. BUSHNELL[1];
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Abstract: Aerobic exercise is widely accepted to confer positive therapeutic effects in persistent pain states. However, it is unclear if the amount of exercise determines the degree of beneficial outcome. Here, we tested whether or not the amount of running exercise is related to the level of exercise-induced analgesia and stress reduction in a rat model of persistent inflammatory pain. Rats (n=12/group) were either sedentary or given access to a running wheel for three weeks (2hrs/day, 4days/wk) following intra-articular injection of Complete Freund's Adjuvant (CFA) or sham injection. We assessed weight bearing capacity of ipsilateral and contralateral hind paws, plasma corticosterone and the level of running exercise. At three weeks post-inflammation, sedentary rats continued to exhibit significant hypersensitivity, with 58.4±6.8% less weight bearing capacity compared to baseline ($p<0.001$), as well as significantly elevated corticosterone levels (1931±421.5pg/ml vs. 838±121.4pg/ml in sham rats; $p<0.05$). On the other hand, access to exercise prevented CFA-induced weight-bearing deficits (97.6±7.4% weight bearing capacity compared to baseline) and maintained plasma corticosterone at sham levels (875±127.7pg/ml vs. 838±121.4pg/ml in sham rats). While the average weekly running distance ranged between 1000-3500m in the CFA-injected group, the amount of exercise was not associated with either weight bearing capacity or corticosterone levels. Our findings suggest that simply engaging in regular exercise, regardless of the level, may be an effective approach to reduce hypersensitivity and stress in persistent pain states.

Disclosures: F. Tarum: None. **M. Pithcer:** None. **R. Imran:** None. **M. Bushnell:** None.

Poster

062. Persistent Pain Treatment

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Program#/Poster#: 62.17/L25

Topic: D.08. Pain

Support: Stryker Corporation research grant

Title: Effects of kilohertz-frequency spinal cord stimulation on conduction in dorsal column axons

Authors: *N. D. CROSBY[1], J. J. JANIK[5], W. M. GRILL[1,2,3,4];
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Abstract: Spinal cord stimulation (SCS) with high frequency pulse trains (~10 kHz) has emerged as a potential treatment for chronic neuropathic pain. However, the effects of kilohertz-frequency stimulation on spinal dorsal column axons and its mechanisms of action remain unknown. The objectives of this work were to quantify the effects of kilohertz-frequency epidural stimulation on action potential conduction of dorsal column axons and to identify thresholds for activation and block of axonal conduction. Male Sprague-Dawley rats were anesthetized with urethane (1.2g/kg) and the spinal cord was exposed at the T10 and T13 vertebrae. A custom platinum electrode was inserted epidurally at T13 to deliver bipolar constant-current stimulation at 10 kHz, using either sinusoidal or biphasic rectangular (24 μs per phase) waveforms. A second pair of contacts on the electrode delivered biphasic 100 μs test pulses to evoke compound action potentials (eCAPs) in the dorsal columns, caudal to the site of SCS. The motor threshold (MT) was identified for each type of SCS, then rats were paralyzed and a recording electrode was placed at T10 to record eCAPs from the dorsal columns before, during, and after 30 second trials of SCS (0.1 mA to 4 mA). MT was 1.0±0.3 mA for sinusoidal and 1.5±0.3 mA for biphasic 10 kHz SCS. Sinusoidal stimulation at 10 kHz significantly reduced eCAP amplitudes beginning at 0.5 mA (0.94-fold below baseline, $p=0.001$) and continuing through 4 mA (0.58-fold below baseline, $p<0.0001$). The reduction in eCAP amplitude at 0.5 mA suggests that conduction block in dorsal column axons began at about 50% MT. Biphasic 10 kHz stimulation from 0.6 to 0.8 mA increased eCAP amplitudes (1.05 to 1.07-fold over baseline $p<0.001$), and eCAP amplitudes did not decline until the SCS amplitude reached 1.5 mA or higher (0.88 to 0.61-fold below baseline $p\leq0.002$). These results support recent reports suggesting that biphasic 10 kHz SCS, which is closer than sinusoidal stimulation to the clinically applied waveform, likely does not attenuate neuropathic pain by blocking dorsal column fibers, because SCS is not administered at the supra-MT amplitudes where block occurred. However, fiber activation or potentiation of firing may occur during 10 kHz SCS, a possibility that warrants further investigation. By establishing thresholds for fiber activation and block in the rat dorsal columns, this work lays the foundation for further *in vivo* investigation of the mechanisms underlying the effects of kilohertz-frequency SCS.

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Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stryker Corporation. **W.M. Grill:** 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.; Stryker Corporation.

Poster

062. Persistent Pain Treatment

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 62.18/L26

Topic: D.08. Pain

Title: Transcranial static magnetic field stimulation for chronic neuropathic pain modulation

Authors: *B. NANDAKUMAR[1], G. H. BLUEMENTHAL[1], A. GRAZIANO[2], G. FOFFANI[3,5], A. OLIVIERO[4], K. A. MOXON[1];
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Abstract: Chronic neuropathic pain (CNP) often arises due to nerve lesion or injury accompanied by maladaptive changes in the peripheral and central nervous system. Chronic neuropathic pain is associated with aberrant cortical reorganization, accompanied by increased excitability in the associated somatosensory-motor cortical representation along with the development of tactile allodynia. Reversal of this maladaptive reorganization using sensory feedback and restoration of defective intracortical inhibition through pharmacological strategies may reduce pain in some patients. However these approaches are often invasive, expensive and accompanied by adverse side effects. It was recently found that that cortical excitability can be modulated in a noninvasive, reversible manner using localized moderate-intensity static magnetic fields generated by Neodymium NdFeB magnets. Transcranial static magnetic field stimulation (tSMS) with exposure of 120 to 200mT over sensorimotor cortical representations transiently reduced the cortical excitability in healthy human subjects. In this study, we assessed the effects of tSMS on pain modulation in a rodent model of chronic neuropathic pain induced by a peripheral nerve injury. Methods: Male Sprague Dawley rats underwent spared nerve injury (SNI) followed by a chronic implant surgery. All these animals either received NdFeB magnet implant (SNI-magnet) or an identical non-magnetic sham implant (SNI-sham) onto the skull. The

implants were placed over the bilateral hind paw representation of somatosensory and motor cortex. Computational finite element modelling was used to estimate the field geometry and design specs of the NdFeB implant based on the field strength requirement of 120mT exposure over the hind limb area. Tactile allodynia was assessed using Von-Frey hair filaments over a period of 4 weeks including a pre-surgical baseline. Result: As expected, animals that received sham magnet treatment developed chronic neuropathic pain as demonstrated by an immediate and severe drop in withdrawal threshold. This drop in withdrawal threshold was attenuated for animals that received magnet therapy. ($F=14.876$, $p<0.05$, $\eta 2=0.788$) Conclusion: These data suggest that tSMS may prevent the onset of the development of CNP after severe nerve injury.

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Poster

062. Persistent Pain Treatment

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Topic: D.08. Pain

Support: DA037673

ICTSI TR000006

IUCRG

Title: Small molecule inhibitors of protein-protein interactions as novel analgesics

Authors: *W.-H. LEE[1,2], Z. XU[3], N. ASHPOLE[4], A. HUDMAN[4], P. KULKARNI[5], G. THAKUR[5], Y. LAI[3], A. HOHMANN[3,6,2];
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Abstract: Abnormal NMDAR activity is linked to central sensitization and chronic pain. This process involves activation of the enzyme neuronal nitric oxide synthase (nNOS) and generation of the signaling molecule nitric oxide (NO). Because the scaffolding protein postsynaptic density 95kDA (PSD95) tethers nNOS to NMDARs, the PSD95-nNOS complex represents a therapeutic target due to its role linking NMDAR activity to NO production. We used a solution binding

assay, AlphaScreen, to quantify potency and efficacy of putative small molecule inhibitors of the PSD95-nNOS complex (i.e. IC87201 and ZL006) in disrupting binding between purified PSD95 and nNOS proteins *in vitro*. We characterized the specificity of PSD95-nNOS disruption by examining abilities of the small molecules to disrupt binding in PSD95-ErbB4 protein pairs. We evaluated the neuroprotective effects of these small molecule inhibitors using a glutamate-induced cell death assay in primary cortical neuronal cultures. We also used inflammatory and a toxic neuropathy models to characterize the antinociceptive efficacies of these small molecules. IC87201 (EC5O: 23.94 μM) and ZL006 (12.88 μM) inhibited PSD95-nNOS binding without altering binding of PSD95 to ErbB4. Thus, IC87201 and ZL006 specifically disrupt PSD95-nNOS interactions but not other PDZ containing protein-protein interactions. Both nNOS-PSD95 inhibitors suppressed glutamate-induced cell death with efficacy comparable to the NMDAR antagonist MK-801. IC87201 and ZL006 preferentially suppressed phase 2A of nociceptive responding in the formalin test and suppressed allodynia induced by intraplantar administration of complete Freund's adjuvant. IC87201 and ZL006 suppressed mechanical and cold allodynia induced by the chemotherapeutic agent paclitaxel (ED50s: 2.47 and 0.93 mg/kg i.p. for IC87201 and ZL006, respectively). However, motor ataxic effects were induced by MK-801 but not by ZL006 or IC87201. Finally, MK-801 produced hyperalgesia in the tail-flick test whereas IC87201 and ZL006 did not alter basal nociceptive thresholds. Our studies establish the utility of using AlphaScreen and purified protein pairs to establish and quantify disruption of protein-protein interactions. Our results demonstrate previously unrecognized antinociceptive efficacy of ZL006 and establish, using two small molecules, a broad application for PSD95-nNOS disruption for treating neuropathic and inflammatory pain. Collectively, our results demonstrate that disrupting PSD95-nNOS protein-protein interactions is effective in attenuating pathological pain without producing motor ataxia associated with NMDAR antagonists.

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Poster

062. Persistent Pain Treatment

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 62.20/L28

Topic: D.08. Pain

Title: The electrophysiological property and anti-hyperalgesic activity of sophoraflavanone G and 6-prenylnaringenin, novel T-type calcium channel blockers

Authors: *S. ONO[1], M. ICHII[1], S. YAMAOKA[1], F. SEKIGUCHI[1], T. FUJITA[1], T. DEGUCHI[1], M. TSUBOTA[1], H. NISHIKAWA[1], S. YOSHIDA[2], K. MURATA[1], H. MATSUDA[1], N. TOYOOKA[3], T. OHKUBO[4], A. KAWABATA[1];
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Abstract: Among three isoforms of T-type calcium channels (T-channels), $Ca_v3.2$ is targeted by hydrogen sulfide, and plays a crucial role in processing of somatic and visceral pain. Selective T-channel inhibitors are thus considered useful for treatment of intractable inflammatory and neuropathic pain. In the present study, we found T-channel inhibitory activity of sophoraflavanone G (SG) purified from SOPHORAE RADIX (SR), a herbal medicine, and also of its analogue, 6-prenylnaringenin (6-PNG), by a patch-clamp assay using HEK293 cells transfected with human $Ca_v3.1$ or $Ca_v3.2$. The IC_{50} values (μM) of SG and 6-PNG were 1.413 and 1.046 for $Ca_v3.1$, and 0.752 and 0.423 for $Ca_v3.2$, respectively, while naringenin, a common core structure of SG and 6-PNG, exhibited no inhibitory activity. In the $Ca_v3.2$-expressing HEK293 cells, SG, but not 6-PNG, shifted the activation curve toward more positive potentials, and 6-PNG, but not SG, shifted the steady-state inactivation curve toward more negative potentials. In the differentiated NG108-15 cells that express high voltage-activated (HVA) calcium channels, SG and 6-PNG at relatively high concentrations suppressed the HVA-currents, the IC_{50} values being 2.4-fold and 3.8-fold higher than those for T-currents in $Ca_v3.2$-expressing HEK293 cells, respectively. SG and 6-PNG even at high concentrations did not suppress high-K-induced contraction in rat aortic smooth muscle preparations. In mice, oral or i.p. administration of SR-extract at 200 mg/kg or intraplantar (i.pl.) administration of SG and 6-PNG at 10 pmol/paw abolished the $Ca_v3.2$-dependent mechanical hyperalgesia/allodynia caused by i.pl. NaHS, a donor of hydrogen sulfide, as assessed by the von Frey test. SG, when administered i.pl. at 10 pmol/paw or i.p. at 2.5-10 mg/kg, suppressed the neuropathic hyperalgesia/allodynia in mice subjected to partial sciatic nerve ligation. SG and 6-PNG, administered i.p. at 10 mg/kg, also suppressed the neuropathic hyperalgesia/allodynia caused by i.p. administration of oxaliplatin, an anti-cancer drug, in mice. Collectively, SG and 6-PNG, novel T-channel inhibitors, appear to have distinct electrophysiological characteristics, and are considered therapeutically applicable to neuropathic pain.

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Poster

062. Persistent Pain Treatment

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Topic: D.08. Pain

Support: Chaire de recherche du canada en neurophysiopharmacologie de la douleur chronique

Title: Interfering with chronic inflammatory pain: the case of Dicer-substrate siRNAs targeting the CCL2/CCR2 chemokine system

Authors: *M.-A. DANSEREAU[1], A. M. JACOBI[2], S. S. ROSE[2], M. A. BEHLKE[2], J.-M. LONGPRE[1], P. SARRET[1];
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Abstract: New developments in the field of small interfering RNA provide neuroscientists with alternative tools to pharmacological agents and genetically modified animals. This may prove especially useful when working with a complexly interacting system such as the highly redundant chemokine family, for which the design of antagonists acting selectively on one receptor target is difficult to achieve. In the current study, we evaluated the efficiency of 27-mers double-strand Dicer substrate small interfering RNA (DsiRNA) targeting the chemokine CCL2 or its main receptor, CCR2, to counteract the nociceptive behaviors associated with the development of chronic inflammatory pain. This animal pain model was used because CCL2 is described as a major mediator of inflammation and pain. DsiRNAs exhibiting different patterns of methylation (either 7 methylation or S3/AS12 methylation) in order to reduce their ability to trigger an innate immune response were first screened *in vitro* for their ability to knockdown CCL2 or CCR2. Real-time quantitative PCR were performed on the RG2 rat glioblastoma cell line, endogenously expressing CCL2 or on HEK cells stably expressing CCR2. The two best leads for CCL2 and CCR2 were then injected intrathecally (i.t.) twice before (-24h and -1h) or after (+1h and +24h) intraplantar administration of complete Freund adjuvant (CFA) into the right hind paw. Mechanical and thermal hypersensitivity, hind paw edema and dynamic weight distribution on each limb were assessed in male Sprague-Dawley rats at days 1, 3, 5 and 7 post-CFA administration. CFA induced a painful hypersensitivity to mechanical and thermal stimuli, a reduction in the weight bore on the ipsilateral hind paw and a major local edema that were all slightly increasing through the experimental period. DsiRNA targeting either CCL2 or CCR2 were able to reduce mechanical hypersensitivity when administered post-CFA. This action was sequence-specific, as one CCL2 duplex failed to reduce painful hypersensitivity, but methylation-independent, as different methylation patterns of the same sequence yielded identical results. Both CCL2 and CCR2 targeting DsiRNA were however unable to prevent any of the pain-associated symptoms measured when administered prior to CFA. In conclusion, we

report that DsiRNAs targeting CCL2 or CCR2 reduce the mechanical hypersensitivity associated with the development of inflammatory pain, and thus constitute promising tools to expand our knowledge of the mechanisms by which the different family of chemokines contributes to chronic pain.

Disclosures: **M. Dansereau:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Integrated DNA Technologies. **A.M. Jacobi:** A. Employment/Salary (full or part-time):; Integrated DNA Technologies Inc. **S.S. Rose:** A. Employment/Salary (full or part-time):; Integrated DNA Technologies Inc. **M.A. Behlke:** A. Employment/Salary (full or part-time):; Integrated DNA Technologies Inc. **J. Longpre:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Integrated DNA Technologies. **P. Sarret:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Integrated DNA Technologies.

Poster

062. Persistent Pain Treatment

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Zhejiang Provincial Natural Science Foundation of China (No. Y2091151, LY14H270016)

China Postdoctoral Science Foundation (No. 2014M550334)

Title: Inhibition of spinal microglia and astrocytes contributes to the anti-allodynic effect of electroacupuncture in neuropathic pain induced by spinal nerve ligation

Authors: ***L. YI**[1], J. DU[1], Y. QIU[2], J. FANG[1], J. LIU[1,2], J. ZHU[3], J. FANG[1];
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Abstract: Besides neurons, activated microglia and astrocytes in the spinal cord dorsal horn (SCDH) contribute to the pathogenesis of chronic pain. Electroacupuncture (EA) has been used widely to treat various chronic pain diseases, however, the underlying mechanisms of EA are still not fully understood. Part 1: Male Sprague-Dawley rats were divided randomly into 4

groups, including an untreated healthy Control group, a True-SNL group that underwent spinal nerve ligation (SNL) and remained untreated, a True-SNL+EA group that underwent SNL followed by EA treatment and a Sham-SNL group that underwent sham surgery and remained untreated. SNL was performed unilaterally at L5 spinal nerve ligation and EA was applied to ST36 and BL60 bilaterally once per day. Part 2: Male SD rats were cannulated for SNL-induced neuropathic pain, and then were randomly divided into dimethyl sulfoxide (DMSO), EA plus DMSO, 4-(4-fluorophenyl)-2-(4-methylsulfonylpheny)-5-(4-pyridyl)-1H-imidazole (SB203580) and EA plus SB203580 groups. SB203580 (30 nmol/L) was administered 5 min prior to EA treatment. Paw withdrawal thresholds (PWTs) were measured ipsilaterally at different timepoints after ligation. Activation of microglia and astrocytes as well as p38 MAPK phosphorylation (p-p38 MAPK) in the SCDH were examined bilaterally by immunofluorescence staining and concentrations of interleukin-1β (IL-1β) and interleukin (IL-6) were measured in the ipsilateral SCDH by ELISA. SNL significantly decreased PWTs and activated glial cells in the superficial laminae of the ipsilateral SCDH. In rats with SNL, GFAP immunoreactivity peaked at 7d and was maintained until 14d post-ligation, while OX-42 immunoreactivity peaked at 3d and declined gradually. EA significantly alleviated SNL-induced mechanical allodynia. Furthermore, EA reduced microglial activation (OX-42 positive ratios) in the lumbar SCDH at 3d post-ligation and suppressed astrocyte activation (GFAP positive ratios) at all time points observed. Spinal p-p38 MAPK was only co-localized with OX-42 in our study. Intrathecal injection of low dose SB203580 had no influence on PWTs, but inhibited the expression of OX-42 positive cells in bilateral SCDH. EA plus SB203580 synergistically increased PWTs and reduced the expression of bilateral spinal OX-42 near to the normal level. EA stimulation alleviates SNL-induced neuropathic pain, at least in part through inhibition of spinal glial activation. Moreover, inhibition of spinal microglia activation may contribute to the immediate effects of EA analgesia which may be partially associated with the reduced expression of p-p38 MAPK.

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Poster

062. Persistent Pain Treatment

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Topic: D.08. Pain

Support: Pittsburgh Tissue Engineering Initiative

Duquesne University Inaugural Provost's Interdisciplinary Research Consortia Award

Title: Mechanical hypersensitivity reversal with drug-loaded nanoemulsion in a CCI model

Authors: *M. SALEEM[1,2], K. VASUDEVA[1,2], S. K. PATEL[3,2], K. T. HITCHENS[4], A. STEVENS[1,2], J. M. JANJIC[3,2], J. A. POLLOCK[1,2];
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Abstract: Chronic pain is an inadequately managed condition in the clinic. Current treatments are not yet targeting underlying pain mechanisms. We sought to target the neuroinflammatory component in the chronic constriction injury (CCI) rat model of neuropathic pain. The chromic gut used to ligate the sciatic nerve in this model causes inflammation. We have previously reported the targeting and imaging of CCI inflammation with a dual labeled perfluorocarbon (PFC) nanoemulsion. This serves as a tool to label circulating macrophages that accumulate at the site of injury, which can be imaged by Near IR fluorescence of live animals or 19F MRI. Histological studies have confirmed the accumulation of macrophages and inflammatory mediators at the site of injury (Vasudeva et al., 2014. PLoS ONE 9(2). In a related study we have demonstrated that macrophages accumulate the drug loaded nanoemulsion *in vivo* at the site of inflammation leading to changes in macrophage infiltration levels indicating anti-inflammatory effects as measured by molecular imaging (NIR and 19F MRI) (Patel et al., 2015. Clinical Immunology). In the current study, the nanoemulsion was loaded with an anti-inflammatory drug and injected intravenously. In this study, tactile allodynia behavioral testing using Von Frey filaments was utilized and paw-withdrawal thresholds were calculated. This measure infers hypersensitivity to stimuli. Inflammation was assessed by imaging the NIR fluorescence of the accumulated nanoemulsion in live animals and separately in post-mortem histological studies. Drug delivered in the infiltrating immune cells results in a significant relief from hypersensitivity associated with tactile allodynia. Drug delivered independently of the nanoemulsion (free drug) has no such effect. These findings demonstrate that PFC nanoemulsion therapy targeted to the inflammatory components of pain is an effective theranostic tool: one that can track and reverse inflammation, resulting in chronic pain relief.

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Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.01/L32

Topic: D.08. Pain

Title: Adult-onset deletion of Nav1.7 in conditional knockout mice

Authors: *S. D. SHIELDS, R. M. REESE, L. DENG, K. SCEARCE-LEVIE, D. H. HACKOS; Neurosci., Genentech, South San Francisco, CA

Abstract: Human genetics strongly implicates the voltage-gated sodium channel Nav1.7 in the function of nociceptors of the peripheral nervous system: loss-of-function mutations in the scn9a gene that encodes Nav1.7 result in congenital insensitivity to pain (CIP), a serious condition in which affected individuals report no experience of pain of any kind in any part of the body and often suffer severe injuries as a result of the loss of pain's protective function. Because null mutation of scn9a in mice results in neonatal lethality, preclinical animal models to study Nav1.7 function have depended heavily on conditional knockout lines in which Cre recombinase, under the control of various promoters, has been used to delete Nav1.7 from different neuronal subpopulations. In many of these cases, incomplete deletion of Nav1.7 from dorsal root ganglion (DRG) neurons has resulted in residual sensitivity to noxious stimuli. Moreover, in both human CIP individuals and knockout mice, deletion of the channel is constitutive beginning during embryonic development. As a result, it has been difficult to predict the expected action of a drug that inhibits Nav1.7 when taken in adulthood by pain patients. A number of important questions still remain: 1) Are all modalities of acute pain affected by complete loss of Nav1.7 in animal models? 2) If Nav1.7 function is interrupted in adulthood, is the effect on pain the same as when it is deleted throughout development? 3) In the setting of established chronic pain, what is the effect of removing Nav1.7? To address these questions, we generated mice with a tamoxifen-inducible deletion of Nav1.7, namely pCAGG-CreERTtg/- x scn9aloxP/loxP (Nav1.7 cKO) mice. We report that both scn9a mRNA and Nav1.7-like immunoreactivity are reduced to undetectable levels in DRG of tamoxifen-dosed Nav1.7 cKO mice, indicating the completeness of deletion in primary afferent somatosensory neurons. After tamoxifen administration, Nav1.7 cKO mice perform similarly to control littermates in a battery of neurological assessments. However, tamoxifen-dosed Nav1.7 cKO mice develop a profound insensitivity to acute noxious stimuli that encompasses heat, mechanical, and chemical stimuli, and both cutaneous and visceral afferents. Our evidence suggests that Nav1.7 is necessary for the ongoing function of the pain sensory nervous system in adulthood, and that drugs that selectively inhibit this channel are likely to be effective in multiple modalities of pain.

Disclosures: S.D. Shields: A. Employment/Salary (full or part-time):; Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genentech. **R.M. Reese:** A. Employment/Salary (full or part-time):; Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genentech. **L.**

Deng: A. Employment/Salary (full or part-time):; Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genentech. **K. Scearce-Levie:** A. Employment/Salary (full or part-time):; Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genentech. **D.H. Hackos:** A. Employment/Salary (full or part-time):; Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genentech.

Poster

063. Pain Models: Behavior I

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Topic: D.08. Pain

Support: NIH Grant NS081707

NIH Grant GM007067

Title: Fully implantable wireless microLEDs for optogenetic control of pain circuitry

Authors: *M. PULLEN-COLON[1], S.-I. PARK[2], D. S. BRENNER[1], G. SHIN[2], C. D. MORGAN[1], B. A. COPITS[1], H. CHUNG[2], K. NOH[2], S. DAVIDSON[1], S. OH[3], J. YOON[2], K.-I. JANG[2], V. K. SAMINENI[1], M. NORMAN[1], J. G. GRAJALES-REYES[1], S. K. VOGT[1], T. KIM[2], M. C. MONTANA[1], J. P. GOLDEN[1], M. R. BRUCHAS[1], J. A. ROGERS[2], R. W. GEREAU, IV[1];
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Abstract: Chronic pain is a debilitating condition that is poorly managed by current therapeutics due to a lack of efficacy and a multitude of side effects. New approaches to manage chronic pain are desperately needed. Optogenetics affords the opportunity to nonpharmacologically control neuronal activity in pain pathways using light. Implementation of optogenetics for pain control in a clinical setting will require both a light-activateable ion channel and a light source that is small and compatible with biological tissues. Hence, we have developed a fully implantable, wirelessly powered, microscale-LED (mLED) devices for targeted light delivery to primary afferent fibers at the level of the sciatic nerve and spinal cord, to illuminate opsin-expressing sensory neurons. We hypothesize that optical stimulation of conditionally expressed opsins modulates the activity of nociceptive fibers and is sufficient for a modified nocifensive response to a noxious stimulus.

To test our hypothesis, we characterized two transgenic mouse lines expressing the excitatory ion channel Channelrhodopsin (ChR2-EYFP) in specific subsets of Advillin-Cre+ and TRPV1-Cre+ primary afferent fibers. We performed immunohistochemistry to confirm selective expression of opsins in nociceptors and patch-clamp electrophysiology to assess the modulation of neuronal activity *in vitro* as a result of photostimulation. We also performed behavioral assays using our implantable mLED devices and found that optical stimulation of primary afferent fibers expressing ChR2-EYFP is sufficient to induce nocifensive behaviors. With this novel wireless technology, mice expressing ChR2-EYFP exhibited aversion to optical stimulation as demonstrated by using a real-time place aversion assay. The development and implementation of these tools will allow us to further dissect the specific roles of molecularly defined sensory fibers, which could lead to novel optogenetic treatments for chronic pain.

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Poster

063. Pain Models: Behavior I

Location: Hall A

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Topic: D.08. Pain

Support: NINDS Grant R01NS042595

Title: Non-reflexive measures of persistent pain in mice

Authors: ***T. SHEAHAN**, E. R. SIUDA, R. W. GEREAU, J. P. GOLDEN;
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Abstract: Chronic pain represents an immense clinical problem, with over 100 million Americans afflicted and an annual price tag exceeding half a trillion dollars. One of the major

hurdles to effective translation of pain research findings is the lack of research that directly translates from animal models into human studies. There has recently been some discussion regarding the validity of using only reflex/withdrawal measures of pain in animal studies, as these endpoints may not adequately model the complexities of human pain. The development of non-reflexive assays that measure non-evoked pain, as well as the affective components of pain, is therefore an important goal that may be useful in increasing the broad translatability of preclinical findings. Voluntary wheel running has recently been demonstrated as a sensitive and reliable endpoint for evaluating inflammation-induced pain. Further, pain has been shown to alter social interactions in rodents. We tested the utility of voluntary wheel running and social interaction assays as non-reflex/withdrawal measures of pain in the context of inflammatory and nerve injury induced-pain on adult C57BL6/J male mice. We report that inflammatory but not nerve injury-induced pain depresses both voluntary wheel running and social interactions compared to control mice, suggestive that these non-reflex/withdrawal assays are most reliable in the context of inflammation-induced pain.

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Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: D.08. Pain

Title: The role of the lateral habenula in the affective dimension of pain

Authors: *M. M. WHITE, S. A. MORRIS BOBZEAN, C. A. SALCIDO, L. I. PERROTTI, P. N. FUCHS;
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Abstract: The lateral habenula (LHb) integrates input from cortical affective regions to aid in decision-making. Additionally, the LHb constitutes an important relay center in a descending pain modulatory circuit involving both the nucleus accumbens and periacqueductal gray. These two functions of the LHb indicate its importance in decision-making during heightened states of arousal (i.e., pain). Here, we tested the hypothesis that cells of the LHb become activated during the Place Escape/Avoidance Paradigm, a test of pain affect in which animals must decide to avoid or withstand painful stimuli. 144 adult experimentally naïve female Sprague-Dawley rats were randomly assigned to one of four test conditions: a no test control group, a mechanical paw withdrawal threshold (MPWT) test group, a Place Escape/Avoidance (PEAP) test group, and a

group that underwent both MPWT and PEAP tests. Additionally, animals within those four conditions were further randomly assigned to receive subcutaneous injections into the left hindpaw of one of three solutions: 0.5% or 2% carrageenan dissolved in 0.9% saline or an equivalent volume saline. Behavioral testing occurred three hours after injection in accordance with previously established maximum behavioral responding. Animals were sacrificed at the conclusion of testing via intracardiac perfusion and brains sectioned and stained via immunohistochemical methods for cFos to evaluate neuronal activity in the LHb. Overall, behavioral results indicated that animals injected with both doses of carrageenan (0.5% and 2%) had significantly lower pain thresholds compared to animals injected with saline. Additionally, pain thresholds in the 2% carrageenan group were significantly lower than in the 0.5% group. Moreover, during PEAP testing, animals injected with carrageenan actively escaped and avoided stimulation to the inflamed left hindpaw significantly more than saline controls. However, no significant difference in avoidance behavior was observed between the carrageenan treatment groups (0.5% and 2%). Preliminary results of immunohistochemical analyses indicated that carrageenan treatment (0.5% and 2%) increased the number of cFos positive cells in the LHb as compared to saline controls. The aim of this study was to assess neuronal activation in the LHb during affective tests of pain in comparison to more basic sensory testing. While the assertion has been made that the LHb is involved in the affective component of pain, this claim has not been directly tested. This research holds importance because it offers further insight into the mechanisms that underlie the pain experience.

Disclosures: M.M. White: None. **S.A. Morris Bobzean:** None. **C.A. Salcido:** None. **L.I. Perrotti:** None. **P.N. Fuchs:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.05/L36

Topic: D.08. Pain

Title: Assessing contending motivational drives of hunger and pain in an operant multi-approach avoidance paradigm

Authors: *C. A. SALCIDO[1], A. L. HARRIS BOZER[1], C. T. MCNABB[3], M. M. WHITE[1], P. N. FUCHS[2];
[1]Psychology, [2]Psychology and Biol., Univ. of Texas at Arlington, Arlington, TX; [3]NIH, Bethesda, MD

Abstract: Pain has been described as a homeostatic emotion that motivates an organism to maintain internal stability. In small amounts, pain can be beneficial and provides critical feedback about bodily functioning. However, when pain is inescapable, it begins to contend with other homeostatic drives, like hunger. To test the hypothesis that pain contends with satiation and attention, we developed a series of experiments that allowed a rat to either satisfy hunger or avoid noxious stimulation in a novel operant approach-avoidance paradigm. In the first experiment, animals were presented with a two lever operant conflict where in order to receive food, they had the choice to press a lever associated with stimulation to a paw with painful inflammation by carrageenan injection or neuropathy by L5 nerve ligation. Results revealed there was no preference for levers/paw stimulation and there was an overall suppression of reward seeking behavior. In addition, control animals with only one pain condition also did not indicate a preference. Due to a possible generalization affect due to spatial proximity of the levers, levers were moved to opposite sides of the chamber for experiment two. Results also suggested that on day one, the generalization effect remained. On day two, there was an emerging preference for stimulation in the non-noxious paw. It was hypothesized that the paradigm may be too complex to allow rodents to indicate their preferences. Therefore, in experiment three, the paradigm was reduced to a single lever, single paw pain inflammation model. There were significant differences between carrageenan and saline groups in mean latency to lever-press, as well as the percentage of trials yielding lever-presses. Even more, when animals were presented with only one pain condition and one lever, this suppression of reward seeking behavior suggested that motivation to avoid pain superseded the motivation to alleviate hunger. Therefore, the results of these studies revealed that suppression of reward seeking behavior occurred only when animals successfully differentiated lever pressing with noxious paw stimulation, and this suppression revealed pain was the most averse contending motivational drive. Future research should be conducted to elucidate the complexity of motivational drives that influence decision-making when pain is present and to investigate the neural mechanisms underlying approach-avoidance conflicts. Utilization of approach-avoidance paradigms such as this can allow researchers to unravel the complexities of the pain experience with the goal of enhancing translation to clinical efficacy.

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Poster

063. Pain Models: Behavior I

Location: Hall A

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Program#/Poster#: 63.06/L37

Topic: D.08. Pain

Title: Spinal cord stimulation in an ovine model of neuropathic pain measured through von Frey filaments, gait analysis, and dorsal horn recordings

Authors: *J. W. MILLER[1], C. G. REDDY[1], S. WILSON[1], B. D. DALM[1], S. SAFAYI[6], K. ABODE-IYAMAH[1], S. K. SHIVAPOUR[6], S. VILJOEN[1], D. C. FREDERICKS[2], K. N. GIBSON-CORLEY[3], N. M. GROSLAND[7], N. U. JERATH[4], K. STONER[7], R. REALE[1], H. OYA[1], N. D. JEFFERY[6], T. J. BRENNAN[5], G. T. GILLIES[8], M. A. HOWARD, III[1]; [1]Neurosurg., [2]Orthopaedic Surgery, [3]Pathology, [4]Neurol., [5]Anesthesia, Univ. of Iowa Hosp. and Clinics, Iowa City, IA; [6]Iowa State Univ., Ames, IA; [7]Biomed. Engin., Univ. of Iowa, Iowa City, IA; [8]Mechanical and Aerospace Engin., Univ. of Virginia, Charlottesville, VA

Abstract: We have developed an ovine model of neuropathic pain intended for use in investigating the analgesic efficacy and possible mechanisms of spinal cord stimulation (SCS). Data were collected from three adult female Polypays sheep. Chronic constriction, i.e., 25% reduction in diameter, of the peroneal nerve produces quantifiable behavioral and neurophysiological responses, without the deficits associated with complete ligation, which seem to be modulated by SCS. In particular, we observed that epidural stimulations from 0.1 to 0.5 V result in an apparent increase in tolerance to von Frey filaments applied to the affected limb and recovery to near-normal gait characteristics during treadmill ambulation. Preliminary evidence from gait analysis suggests an acclimation to the stimulation signal within < 150 steps on the treadmill. In parallel with this, we have developed neurophysiological methods for obtaining single unit recordings from dorsal horn neurons in order to characterize the effects of chronic pain and SCS on spontaneous activity. The development of a large-animal model of neuropathic pain and spinal cord stimulation is a crucial step towards improving clinical care.

Disclosures: J.W. Miller: None. **C.G. Reddy:** None. **S. Wilson:** None. **B.D. Dalm:** None. **S. Safayi:** None. **K. Abode-Iyamah:** None. **S.K. Shivapour:** None. **S. Viljoen:** None. **D.C. Fredericks:** None. **K.N. Gibson-Corley:** None. **N.M. Grosland:** None. **N.U. Jerath:** None. **K. Stoner:** None. **R. Reale:** None. **H. Oya:** None. **N.D. Jeffery:** None. **T.J. Brennan:** None. **G.T. Gillies:** None. **M.A. Howard:** None.

Poster

063. Pain Models: Behavior I

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Topic: D.08. Pain

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Title: A novel platform for genetic dissection of nociceptive sensitization to cold

Authors: *H. N. TURNER[1,2], E. SUMNER[1,3], C. LANDRY[4], M. GALKO[1];
[1]Genet., MD Anderson Cancer Ctr., Houston, TX; [2]Neurosci. Program, Univ. of Texas Grad. Sch. of Biomed. Sci. at Houston, Houston, TX; [3]Univ. of Texas Grad. Sch. of Biomed. Sci. at Houston, Houston, TX; [4]ProDev Engin., Missouri City, TX

Abstract: Organisms from flies to mammals utilize thermoreceptors to detect and respond to ambient and noxious thermal stimuli. Numerous clinical conditions disrupt the sensory machinery, such as in patients suffering from tissue damage (from a wound or sunburn), or damage to the peripheral nerves, common in patients undergoing chemotherapy treatment, or suffering from neurodegenerative disorders. Our goal is to dissect the mechanisms underlying nociceptive sensitization using the genetically tractable *Drosophila* species, as a model. Larvae respond to a high temperatures and harsh mechanical stimuli with a 360° lateral body roll (BR) through class IV multiple dendritic peripheral sensory neurons that innervate the epidermis along the entire body length of the larva. This behavior sensitizes to heat after UV damage to the larval epidermis. Here we show that larvae are also sensitized to cold stimuli after similar epidermal injury. Using a novel "cold probe" assay we found that *Drosophila* larvae produce a mutually exclusive set of primary reactive behaviors, distinct from the commonly reported aversive "corkscrew" behavior seen in response to the high temperature probe. These behaviors include a posterior raise (PR), a combined head and tail raise into a U-Shape (US), and a full-body contraction (CT) behavior. These behaviors are cold specific, occurring in 60% of larvae below 12º C, and require sensory neurons different from those required for responding to heat. After UV exposure, larvae exhibit an increase in the US and also exhibit the typically heat-evoked aversive rolling behavior in response to the cold probe. Larvae simultaneously show a decrease in the CT and TR responses. We see similar results when the epidermis is injured via a pinch wound, which damages the epidermal cell layer and nociceptive neurons while leaving the underlying cuticle intact. These findings suggest a switch in the motor program selected to respond to a cold stimulus after epidermal injury. We have established the first system to study noxious cold responses and cold nociceptive sensitization in *Drosophila*. Our unique tools and assays should allow us to further uncover the conserved molecular and genetic bases of cold nociceptive sensitization, including the cells and channels required for this process.

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Poster

063. Pain Models: Behavior I

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Title: Persistent thermal allodynia evoked by nerve growth factor (NGF) is mediated by TRPV1 and oxidative mechanisms

Authors: *M. ESKANDER[1], S. RUPAREL[1], P. CHEN[1], X. GAO[2], K. HARGREAVES[1]; [1]Endodontics, [2]Biochem., UTHSCSA, San Antonio, TX

Abstract: Chronic pain management remains a major health care problem due to an incomplete understanding of mechanisms involved in the transition to persistent pain states. Nerve growth factor (NGF) is a sufficient stimulus in humans to trigger persistent pain, but the mechanisms mediating this transition are unknown. We have tested the hypothesis that NGF evokes a persistent nociceptive state via increased TRPV1 activity and oxidative mechanisms. Using blinded observers, rats received NGF (30 μg/kg, s.c.) for 5d and then tested for thermal allodynia using the radiant heat test. NGF evoked a persistent thermal allodynia that extended up to 11d. Thermal allodynia was significantly and dose-dependently reversed by intraplantar (i.pl.) injection of the TRPV1 antagonist capsazepine (CPZ), the cytochrome p450 (CYP) and lipoxygenase (LOX) inhibitor and antioxidant nordihydroguaiaretic acid (NDGA), and the phospholipase A2 (PLA2) inhibitor bromoenol lactone (BEL), suggesting that persistent allodynia is mediated by TRPV1, oxidation, and PLA2-mediated release of free fatty acids. NGF treated animals showed increased nocifensive responses to intraplantar capsaicin (1μg, $p<0.001$), therefore we utilized whole cell patch clamp to conduct a capsaicin concentration response in acutely cultured lumbar DRG cell bodies. Maximal current density (pA/pF) was enhanced after NGF treatment ($p<0.05$) with no change in EC50 values, suggesting increased membrane density of TRPV1. Similarly, maximal capsaicin-evoked calcitonin gene related peptide (CGRP) release

from hind paw skin was enhanced after NGF ($p<0.05$) with no change in EC50 values, suggesting similar effects on TRPV1 between DRG cell bodies and nerve terminals in skin. Finally, we used HPLC/MS to demonstrate that NGF significantly increased concentrations of oxidized TRPV1-active lipids in skin, which were attenuated by i.pl. injections of NDGA and BEL. Collectively, these data support the hypothesis that NGF-induced persistent nociception is mediated by PLA2-dependent generation of oxidized lipids leading to activation of TRPV1. Since NGF is implicated in many chronic pain disorders, increased understanding of the oxidative mechanisms mediating persistent nociception may advance the development of novel analgesics.

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Poster

063. Pain Models: Behavior I

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Topic: D.08. Pain

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Title: Psychosocial stress and susceptibility to chronic postsurgical pain in the rat

Authors: *V. ARORA, C. E. MORADO-URBINA, C. ASCHENBRENNER, K. HAYASHIDA, T. J. MARTIN, J. C. EISENACH, C. M. PETERS;
Dept. Of Anesthesiol., Wake Forest Univ. Sch. of Hlth. Sci., Winston Salem, NC

Abstract: Chronic postsurgical pain (CPSP) is a frequent and often disabling complication of many surgical procedures. Identification of patients at risk of developing CPSP remains inadequate. Analysis of predictive and pathologic factors is important to develop rational strategies to prevent development of CPSP. Clinical studies have suggested that psychosocial and socio-environmental factors are associated with the development of CPSP, however few animal studies have examined this relationship. The goal of this study was to investigate the impact of preoperative psychological stress on postoperative recovery following surgery in the rat. Psychosocial stress was induced in male Sprague-Dawley rats by two weeks of repeated social defeat (10 sessions) using the resident intruder paradigm. Rats exposed to social defeat

had reduced weight gain and reduced plasma corticosterone levels compared to non-defeated rats. Behaviorally defeated rats did not display anxiety like behavior based on the elevated plus maze and open field test but did show increased immobility time on the forced swim test indicating depression like behavior. Social defeat stress also induced a transient mechanical hypersensitivity and spinal microglial activation compared to non-defeated rats. Three days after the last session of social defeat, rats had plantar incision surgery. Mechanical withdrawal thresholds were measured for several weeks using von Frey filaments and individual postoperative trajectories were ¬modeled using mixed growth curve analysis. Preoperative psychosocial stress significantly delayed resolution of mechanical hypersensitivity for several weeks following surgery evident as a reduced slope of recovery. Preoperative measures of affective distress including stress induced hypersensitivity, immobility time during FST, plasma corticosterone levels were correlated to postoperative outcomes to identify potential predictors of impaired recovery. The results indicate that the stress of social defeat can produce physiological and behavioral outcomes which reflect aspects of risk for the development of CPSP. Future studies will investigate mechanisms behind the association between affective distress and chronic pain after surgery and test targeted interventions based on these mechanisms.

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Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.10/L41

Topic: D.08. Pain

Title: Effects of chronic pain on hippocampal microglia and development of depressive-like behaviors

Authors: M. CARDER, B. LAMB, L. SEMKE, M. LEONG, M. LENDE, 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. Thus, in this study, we examined the effects of persistent pain on activation of immune-inflammation processes in the hippocampus. 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 throughout the 42 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 activation of interlukin-1-beta (IL-1ß)-mediated inflammatory mechanisms, as well as negative regulation of MAPK signaling. These results resemble previous findings linking stress-induced IL-1ß up-regulation and suppression of neurogenesis in the adult rat hippocampus and, thus, may present novel factors contributing to the depressive-like behaviors observed in chronic pain models. Furthermore, studies are currently underway to characterize pain-mediated alterations on the microglial cell number, size and activation level within the hippocampus. Together these studies 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.

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Poster

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Program#/Poster#: 63.11/L42

Topic: D.08. Pain

Title: Comparison of the analgesic effects of amphetamine and caffeine on tail-flick nociception following repeated forced exercise

Authors: S. PIERCE, L. PEREZ, L. MININBERG, *J. A. SCHROEDER;
Dept Psychol, Connecticut Coll, New London, CT

Abstract: Caffeine and amphetamine have been used by athletes to enhance performance and resist fatigue. Both stimulants have also been shown to be an effective analgesics or analgesic adjuvants. In the current study, for ten consecutive days, rats received caffeine (5, 10 or 20 mg/kg, ip), amphetamine (1.0, 2.5 or 5.0 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. Both stimulants significantly

enhanced active swim time vs. passive floating. Exercise alone significantly enhanced analgesia following forced exercise, however the effect dissipated by 15 minutes following exercise. Caffeine alone at all doses did not produce an antinociceptive effect, however 10 or 20 mg/kg caffeine administered prior to forced swimming significantly enhanced exercise-induced analgesia. Amphetamine alone produced a dose dependent analgesic effect and similar to caffeine, significantly enhanced exercise-induce analgesia. Tolerance to either drug's effects on exercise-induced analgesia was not observed. These results suggest that repeated forced exercise alone is antinociceptive, an effect that is enhanced by psychostimulants with separate pharmacologic profiles.

Disclosures: **S. Pierce:** None. **L. Perez:** None. **L. Mininberg:** None. **J.A. Schroeder:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.12/L43

Topic: D.08. Pain

Title: Pain-related behaviors in nonhuman primate models of knee osteoarthritis

Authors: ***S. NEMOTO**[1], S. OGAWA[2], Y. AWAGA[2], M. TAKASHIMA[2], K. SUEHIRO[2], T. KAMADA[2], A. HAMA[2], A. MATSUDA[2], H. TAKAMATSU[2], K. UMEMURA[3];
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Abstract: The social and economic burdens of osteoarthritis (OA) are expected to rise as the global population ages. Treatments to reverse tissue damage as well as to alleviate pain are needed. Much of the pathophysiology of OA has been derived from preclinical rodent OA models, but a potential limitation is their phylogenetic distance from humans. Thus, nonhuman primate (NHP) models of OA were developed. Methods designed specifically for the NHP were also developed to quantify pain-related behaviors, including weight bearing, gait disturbance and knee pressure threshold. Following intraarticular injection of monoiodoacetate (MIA), increased knee joint skin temperature and knee joint diameter were observed, suggestive of inflammation. While knee joint temperature remained elevated, knee joint diameter diminished over time. After MIA-injection, significantly decreased weight bearing of the ipsilateral limb, gait disturbance, characterized by diminished mobility of the ipsilateral limb, and decreased pressure threshold, suggestive of pressure "hyperalgesia," were observed over time in the ipsilateral but not

contralateral knee joint. Daily treatment with diclofenac prevented the full expression of pain-related behaviors following MIA injection over time compared to vehicle-treated controls. At 36 days post-MIA injection, an acute injection of morphine transiently reversed pain-related behaviors. Following ipsilateral medial meniscectomy (MMx), weight bearing and knee pressure threshold were normal. Gait disturbance was not observed. However, following an exercise schedule, decreased weight bearing, gait disturbance and pressure hyperalgesia emerged. An analgesic dose of morphine decreased pain-related behaviors. Cartilaginous erosion was observed with magnetic resonance imaging in the ipsilateral knee. Interestingly, pain and decreased functioning are seen with activity but not during periods of inactivity following MMx, similar to clinical OA. The current NHP OA models demonstrate clinical symptoms of knee OA and could highly useful in further elaborating disease mechanism and testing treatments that both ameliorate symptoms and reverse disease.

Disclosures: **S. Nemoto:** A. Employment/Salary (full or part-time):; Hamamatsu Pharma Research, Inc. **S. Ogawa:** A. Employment/Salary (full or part-time):; Hamamatsu Pharma Research, Inc. **Y. Awaga:** A. Employment/Salary (full or part-time):; Hamamatsu Pharma Research, Inc. **M. Takashima:** A. Employment/Salary (full or part-time):; Hamamatsu Pharma Research, Inc. **K. Suehiro:** A. Employment/Salary (full or part-time):; Hamamatsu Pharma Research, Inc. **T. Kamada:** A. Employment/Salary (full or part-time):; Hamamatsu Pharma Research, Inc. **A. Hama:** A. Employment/Salary (full or part-time):; Hamamatsu Pharma Research, Inc. **A. Matsuda:** A. Employment/Salary (full or part-time):; Hamamatsu Pharma Research, Inc. **H. Takamatsu:** A. Employment/Salary (full or part-time):; Hamamatsu Pharma Research, Inc.. **K. Umemura:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.13/L44

Topic: D.08. Pain

Support: Brody School of Medicine (SC)

Title: Spinal dopamine / morphine interactions in the D3 receptor knockout animal model of Restless Legs Syndrome (RLS)

Authors: A. P. YLLANES[1], S. SAMIR[1], K. L. BREWER[2], *S. CLEMENS[1];
[1]Physiol., [2]Emergency Med., East Carolina Univ., Greenville, NC

Abstract: Restless Legs Syndrome (RLS) is an aging-related sensorimotor disorder characterized by abnormal limb sensations that worsen at rest and that severely disrupts sleep. Primary treatment is directed at CNS dopamine (DA) systems that activate D2-like receptors (in particular, D3 and D2). However, long-term therapy directed at these receptors can lead to augmentation, which then routinely leads to a switch to an opioid treatment plan. We recently reported that a dysfunction of the D3 receptor (D3R) system was associated with a lack of responsiveness to morphine as well as increases in D1 receptor (D1R) protein levels in the spinal cord, and that block of D1Rs can reduce spontaneous locomotor activity. As D1Rs and D3Rs can form functional heterodimers, we hypothesized that a modulation of the D1R system might provide a novel means by which to prevent the development of D3R agonist-induced augmentation and the lack of responsiveness to morphine. We tested thermal pain withdrawal latencies over the 2-year life span of male wild type (WT) and D3R knockout mice (D3KO) with varying dosages and/or combinations of morphine, D3R agonist, and D1R antagonist. After establishing baseline withdrawal latencies, animals were treated with morphine (2 mg/kg and 5 mg/kg, respectively), D3R agonist (0.5 mg/kg), D1R antagonist (0.1 mg/kg), and morphine-D1R antagonist combinations (2 mg/kg + 0.1 mg/kg and 5 mg/kg + 0.1 mg/kg, respectively). We found that application of the D3R agonist increased withdrawal latencies in WT but had no effect in D3KOs. Low morphine increased pain withdrawal latencies in young and old WT animals, but had no effect in young or old D3KO. In contrast, high morphine increased withdrawal latencies in both 2 months- and 2 years-old animals. Blocking D1R function alone did not alter responses in young or old WT, or old D3KO; however, it increased withdrawal latencies in young D3KO. Combination treatments of D1R block with low morphine increased latencies in young and old WT, and young but not old D3KO. Interestingly, combination of D1R block and high morphine increased latencies in young and old WT and D3KOs. These data suggest that D1R-D3R interactions mediate morphine responsiveness in an aging-dependent manner, and that block of D1R function can restore opioid sensitivity in an animal model of RLS with D3R dysfunction.

Disclosures: **A.P. Yllanes:** None. **S. Samir:** None. **K.L. Brewer:** None. **S. Clemens:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.14/M1

Topic: D.08. Pain

Title: Validation of the kaolin/carrageenan model as an experimental model of osteoarthritis in rats

Authors: *F. PINTO-RIBEIRO[1,2,3], D. AMORIM[3,2], A. DAVID-PEREIRA[3,2], A. LIMA[3,2], R. NOGUEIRA[3,2], N. SEVIVAS[3,2], A. ALMEIDA[3,2];
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Abstract: Introduction: Human osteoarthritis (OA) is a chronic degenerative disorder affecting joints, most frequently the knee. Many experimental models have been developed in several species attempting to reproduce features of the human OA but so far the available models correlate poorly with the human pathogenesis in terms of anatomy, functionality and cartilage thickness and repair. Recently, the kaolin and carrageenan (K/C) model was shown to mimic the acute inflammatory phase of OA, reproducing the main features of the onset and initial stages of human OA. Nonetheless, no study has yet shown the time-dependent changes observed in articular structures after the induction of this experimental model. Purpose: To evaluate changes in nociceptive behaviour and gait in male and female rats with experimental OA through a period of six weeks; and to perform a time dependent characterization of knee joint pathophysiological and radiological alterations. Materials and Methods: Hence, in the work herein, right knee K/C-induced OA in adult male and female wistar han rats was evaluated through a period of six weeks in terms of nociceptive behaviour, gait and radiological and histological alterations of articular structures.Comparisons between groups were performed using unpaired t-tests or the non-parametric Mann-Whitney test. A two-way ANOVA was used to assess changes in weight gain between experimental groups. Comparisons between the results of the OA grading scale were performed using Pearson analysis and data reliability was evaluated using the Cronbach alpha test. Results: Our data showed increased ipsilateral oedema and mechanical hyperalgesia in arthritic rats. Radiological and histological findings demonstrated a significant narrowing of the articular space, the development of osteophytes, bone remodelling and chondrocyte hypertrophy and disorganization. Discussion and Conclusions: Our findings support the use of the K/C model as a valid animal model for the preclinical study of OA as its shares important time-dependent homologies in what concerns nociception, gait and articular degeneration with the human pathology.

Disclosures: F. Pinto-Ribeiro: None. **D. Amorim:** None. **A. David-Pereira:** None. **A. Lima:** None. **R. Nogueira:** None. **N. Sevivas:** None. **A. Almeida:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.15/M2

Topic: D.08. Pain

Support: NIH Grant NS080889

Title: Effects of chemotherapy exposure on pain sensitivity in the developing rat

Authors: *K. A. SCHAPPACHER, M. L. BACCEI;
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Abstract: Recent work points to an age-dependent effect of peripheral nerve damage on pain sensitivity, as neonatal rodents display a significantly delayed onset of mechanical hypersensitivity compared to adults. While high dose chemotherapy consistently evokes neuropathic pain in adults, the degree to which pediatric chemotherapeutic regimens influence nociceptive processing throughout development remains unknown, in part due to the lack of an established animal model of chemotherapy-induced neuropathy during early life. Therefore, the present study investigated the effects of early exposure to vincristine (VNC), commonly used in the treatment of pediatric cancers, on mechanical and thermal pain sensitivity in the developing rat. Sprague Dawley rats received daily i.p. injections of 25, 50, 75, or 100 μg/kg VNC, or equivalent volumes of saline, with a five-day on, two-day off schedule starting on postnatal day (P)10 for a total of 10 injections. Mechanical reflex withdrawal thresholds were measured prior to each injection and then at weekly intervals until eight weeks of age. Withdrawal latencies in response to radiant noxious heat were evaluated weekly starting on P26 until eight weeks of age. VNC at 25 and 50 μg/kg did not significantly alter mechanical or thermal thresholds at any time point compared to saline-treated littermate controls. Since daily administration of 75 and 100 μg/kg VNC resulted in death following the third dose, we modified our dosing protocol and administered 5 i.p. injections of 100 μg/kg vincristine (or saline) every other day starting at P10. Body weights were monitored and mechanical and thermal withdrawal thresholds were measured as described above until eight weeks of age. Although rats routinely survived this VNC dose, both male and female VNC-treated rats had significantly reduced weight gain compared to control groups starting at P33. In both sexes, mechanical and thermal reflex sensitivity in the VNC-treated and saline-treated groups were similar throughout the time period examined. Overall, the present results suggest that the administration of high doses of vincristine during the early postnatal period fails to evoke common signs of neuropathic pain in the developing rat.

Disclosures: K.A. Schappacher: None. **M.L. Baccei:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.16/M3

Topic: D.08. Pain

Title: An over ground testing apparatus for evaluation of spinal cord injury associated neuropathic pain

Authors: *E. A. DUGAN, J. SAGEN;
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Abstract: Spinal cord injury (SCI) is associated with both locomotor deficits and sensory abnormalities that include the development of neuropathic pain. Preclinical research for associated neuropathic pain has depended on the use of behavioral testing that is reflexive in nature, however, there is a growing need for the inclusion of testing paradigms that include a cognitive component to the response. We have designed a locomotor over ground apparatus that has exchangeable floors for examination of developed pain behaviors in response to cold (thermal) and rough (tactile) stimulation. Male SD rats are pre-trained for 4 weeks to cross three different over ground surfaces (standard, cold and rough) at a consistent walking pace for a food reward. To further encourage the rodents to cross the walkway, a light was placed at the start of the runway with a dark box at the other end to take advantage of the innate photophobia in rodents. The outcome measures we examined where 1) time spent in the lighted start chamber prior to initiating the crossing (animals were removed after 3 minutes and the crossing was marked incomplete), 2) time to cross the over ground surface, and 3) number of completed crossings out of the number of attempted crossings. All three surfaces were used during pre-training and testing for this experiment. Crossing times for control animals did not differ on any of the three surfaces. We found an increase in crossing speeds for the cold over ground compared to pre-SCI times which was inversely correlated with the development of cold allodynia (acetone testing). We did not see a change in the number of completed crossing or time spent in the start chamber suggesting the animals choose to cross the apparatus at a faster speed, which did not result in a food reward. We found a decrease in crossing speeds at 2, 6, 7, 10 and 12 weeks post SCI for the rough over ground compared to pre-SCI times which was inversely correlated with the development of mechanical allodynia (Von Frey testing). We also show an increase in the time spent in the start chamber with many animals “timing out” and not attempting to cross the rough surface at post SCI weeks 4-12. We did not see differences in the pre- and post-SCI crossing times for SCI animals on the standard over ground surface suggesting that locomotor deficits are not associated with the changes in crossing speeds observed with cold and rough over ground surfaces. This over ground testing apparatus provides a rapid additional screening test that is easy to establish in animal models for the development of cold and mechanical allodynia.

Disclosures: **E.A. Dugan:** None. **J. Sagen:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.17/M4

Topic: D.08. Pain

Support: Owens Grant

Title: A novel method to study oral cancer pain

Authors: *L. CHODROFF[1], M. BENDELE[2], M. HENRY[2], S. RUPEREL[2];
[1]Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX; [2]Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: Pain is the primary symptom reported by oral cancer patients. Most patients require NSAIDs and opiates that are either only partially effective or develop tolerance and side effects. Because pain occurs even when the tumor is still quite small in size, it is likely that oral cancer cells control the activities of surrounding nociceptors at the site of the tumor. Therefore, it is crucial to understand the mechanisms of tumor-nerve interactions in order to develop more effective ways of treating oral cancer pain. The current study developed a novel tongue cancer pain model that includes *in vivo* nociceptive behavior assays as well as anatomical and expression studies, to study mechanisms of oral cancer pain. The human oral squamous cell carcinoma (OSCC) cell line HSC2 (3.5 x 10^5 cells) or normal cells was injected (50 uL) in the ventral side of the tongue of athymic male mice and after allowing time for growth, nociceptive behavior was measured. Mechanical allodynia was assessed by measuring changes in feeding as well as by application of von Frey filaments to the vibrissal pad. Higher order responses to spontaneous nociception was assessed using a conditioned placed preference (CPP) test where animals were paired with morphine 3mg/.kg for 3 days and then tested for chamber preference on the 4th day. Data were analyzed by ANOVA. Immunohistochemistry was performed on tongue-tumor sections as well as trigeminal ganglia (TG) to anatomically characterize the model. Our results showed that HSC2 mice, but not normal mice, presented reduced feeding by day 7 post-inoculation that was reversed by indomethacin 5mg/kg ($p<0.05$). Similarly, tumor-grown mice showed mechanical hypersensitivity compared to normal mice that was also reversed by indomethacin. CPP testing revealed that HSC2 mice showed increased preference to the morphine-paired chamber compared to normal mice. Additionally, we observed no difference in ATF3 expression in TG of normal and tumor-growing mice suggesting that the tumor did not induce nerve-damage. However, we observed significant CGRP positive innervation within the tumor-tongue sections. In conclusion, the study characterizes a novel oral cancer pain model that

can be used to study the effect of novel analgesics for the treatment of oral cancer pain as well as determine mechanisms by which oral tumor activate the pain transduction pathway.

Disclosures: **L. Chodroff:** None. **M. Bendele:** None. **M. Henry:** None. **S. Ruperel:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.18/M5

Topic: D.08. Pain

Support: NIGMS of NIH Grant SC1NS078778

NIGMS of NIH RISE Grant R25 GM59994

Title: Activation of membrane estrogen receptors rapidly attenuates opioid receptor-like 1 (ORL1) receptor-mediated modulation of nerve injury-induced hypersensitivity in the rat spinal cord

Authors: *D. M. HECKARD, S. S. MOKHA;
Meharry Med. Col., Nashville, TN

Abstract: Numerous studies report that women have a higher prevalence of chronic pain disorders than men. We have previously shown that estrogen attenuates opioid receptor like -1 (ORL1) receptor mediated thermal antinociception in females [Claiborne et al. J Neurosci. 26:13048-53, 2006]; and down regulates the ORL1 gene expression [Flores et al. Neurosci. 118:769-78, 2003]. Recently, we have shown that estrogen activation of membrane estrogen receptors (GPR30, Gq-mer, ER α, but not ER β) abolishes ORL1-mediated acute thermal antinociception via an ERK2-dependent non-genomic mechanism [Small et al. Neurosci. 255:177-190, 2013]. However, the role of membrane estrogen receptors (mERs) in modulating opioid receptor -mediated attenuation of neuropathic pain is unknown. Thus, the present study investigated whether activation of mERs attenuates ORL1-mediated modulation of nerve injury-induced mechanical hypersensitivity. We employed the spared nerve injury (SNI) model as previously described by Decosterd and Woolf [Pain. 87:149-58, 2000] to induce mechanical hypersensitivity in male and OVX female Sprague Dawley rats. Paw withdrawal thresholds (PWTs) were recorded using an automated dynamic plantar aesthesiometer. After a 7-day recovery period, sham and SNI rats were intrathecally administered E2BSA, a membrane impermeable analog of estradiol (E2BSA), or a selective mER agonist immediately followed by

OFQ, the endogenous ligand for the ORL1 receptor, into the lumbosacral spinal cord of rats through an implanted PE-10 cannula. SNI significantly reduced PWTs in both males and OVX females. Intrathecal administration of OFQ significantly increased PWTs in both males and OVX females. E2BSA as well as selective mER activation abolished OFQ-induced increase in PWTs. Thus, we conclude that activation of mERs rapidly attenuates ORL1-mediated modulation of nerve injury-induced mechanical hypersensitivity which provides evidence of a biological mechanism that increases female vulnerability to the development of chronic pain disorders.

Disclosures: D.M. Heckard: None. **S.S. Mokha:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.19/M6

Topic: D.08. Pain

Title: Connotative meaning of the concepts of lumbago and sciatica in patients with chronic lumbar pain through a model of Natural Semantic Network

Authors: R. CORONADO-ZARCO[1], E. CRUZ-MEDINA[2], *I. ZARCO DE CORONADO[4], S. I. MACÍAS-HERNÁNDEZ[3];
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Abstract: A Natural semantic network (RSN)is a series of processes that a person integrate about words, verbal symbols, meanings and references to create productive and generative concepts that should modify individual´s behavior. Patients with low back pain require not only knowledge but also acceptance of their condition in order to comply with treatment, so we considered the identification of the connotative meaning of "lumbago" and "sciatica" with a RSN in patients with lumbar spine pathology. Design: Cross sectional study. Setting: Spine Rehabilitation Service, Mexico City. Participants: A total of 203 surveys were applied to the service patients, 111 women, 93 men with a mean age of 44.4 years. Main Outcome Measures: In the survey were provided in The stimulus "lumbago" and "sciatica" words, and asked patients to relate to other 5 words from arbitrary form and then rank them in order of importance. Frequency of mention (J) was determined, group consensus (C10), semantic memory association, semantic weight (M) and percentage of defining words. **Results**: Value J: 164 for lumbago, 195 for sciatica; C10: 33.1for lumbago and 26.9 for sciatica; Value M: lumbago (Pain 1793, spine

623, back 466, discomfort 405lumbar 264) Sciatica (Pain 1573, nerve 947, leg 560); Median: 14.6 lumbago 18.0/sciatica; Range:9.2-88.2 lumbago / Sciatica 14.7-119 (p = 0.315, u = 36). Lumbago PV / S (Median 14.4/13.8, Rank 10.8-88.3/8.9-86.6 (p = 0.684;U = 44) Sciatica PV / S (Median 7.4/6.9, Rank 8.1-46.1/5.1-42.1 (p =0.436 , U = 39).**Conclusion**: There is no difference in the conceptualization of the terms. Therefore it is necessary to emphasize patient´s education with the modification of these concepts to promote behavioral changes that improve adherence to treatment

Disclosures: R. Coronado-Zarco: None. **E. Cruz-Medina:** None. **I. Zarco de Coronado:** None. **S.I. Macías-Hernández:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.20/M7

Topic: D.08. Pain

Title: Post-ganglion spinal nverve injury causes more severe radiculopathy: from humans to animal studies

Authors: *J.-H. LIN[1,3,2,4,5], Y.-W. YU[3], C.-C. CHEN[4,5], Y.-H. CHIANG[3,2];
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Abstract: Introduction: Lumbar spinal stenosis (LSS) resulting in lumbar radiculopathy can be anatomically classified as central lumbar spinal stenosis (CLSS) and lateral lumbar spinal stenosis (LLSS). CLSS comprises the preganglion part of the spinal nerve, while LLSS comprises the ganglionic and postganglionic parts. Lumbar radiculopathy has been reported much more severe in LLSS than in CLSS. Accordingly, we argued it is the site of nerve injury that determines the severity of lumbar radiculopathy. The aims of this study is to compare the sensory phenotype and pain scores between patients with CLSS or LLSS and to compare the pain behaviors between animals with the pre- or post-ganglionic spinal nerve injury. Materials & methods: The clinical study prospectively included patients who had degenerative spinal disorders on L4/5 or L5/S1 levels with/without unilateral L5 radicular pain. The compressing pathologies on L5 nerve root were divided into 2 categories: central spinal stenosis, or lateral spinal stenosis. The sensory examination included warm, cold, static mechanical, vibration, and

dynamic mechanical sensation on the foot dorsum ipsilateral to the pathology, and the foot dorsum contralateral to the pathology on the images served as the control. The association between the sensory examination and the compression sites was analyzed. Then, we established two animal models of lumbar radiculopathy- pre-ganglion and post-ganglion injury to identify the differences between in terms of von Frey filament test, and incapacitance test for assessing mechanical hyperalgesia and spontaneous pain, respectively. Results: Forty-eight patients met the inclusion criteria. The incidences of compressing pathology were 42% (20/48) with central spinal stenosis and 58% (28/48) with lateral spinal stenosis. There were 54% (26/48) patients with impaired warm sensation, 45% (22/48) with impaired cold sensation, 27.78% (10/36) with impaired static mechanical stimuli, and 39.58% (19/48) with impaired dynamic mechanical stimuli. The presence of sensory deficit was highly associated with lateral spinal stenosis ($p=0.0023$) with 78.5% sensitivity and 65% specificity. In animal study, the postganglion group showed more severe and persisted course of hypersensitivity both in von Frey test and incapacitance test. This phenomenon last at least for 12 weeks. Conclusion: The results of the present study showed patients with LLSS had more severe lumbar radiculopathy. In similarity, our results of animal study also showed animals with post-ganglion injury had more severe hypersensitivity. As a result, the post-ganglionic spinal nerve injury may cause more severe lumbar radiculopathy.

Disclosures: **J. Lin:** None. **Y. Yu:** None. **C. Chen:** None. **Y. Chiang:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.21/M8

Topic: D.08. Pain

Title: Assessment of gait deficiencies to provide a functional endpoint in the spinal nerve ligation model and spared nerve injury model in rats

Authors: A. NURMI, *L. TOLPPANEN, T. HEIKKINEN, R. HODGSON, A.-M. ZAINANA; Charles River Discovery Services, Kuopio, Finland

Abstract: Nerve compression, damage to peripheral nerves or damage to spinal nerves often results in chronic neuropathic pain. There are multiple rodent models to model neuropathic pain, which are widely used in research and drug development to understand the biology/pathology of this condition and also to develop novel therapies to improve quality of life. Two rodent models used for neuropathic pain research, spinal nerve ligation (SNL) and spared nerve injury (SNI),

produce heightened sensitivity to painful stimuli. However, there has been relatively little that has been done to determine the extent to which SNL and SNI impair motor function in rodents. In this presentation we show how tactile and thermal stimuli are observed in these models over chronic follow-up period and how these symptoms respond to clinically used therapies against neuropathic pain. Since we have previously seen and reported gait changes in another type of model containing neuropathic pain symptoms, in neuritis model, we sought to characterize potential gait related changes and phenotypes for both of the models by using kinematic fine motor analysis. Sprague-Dawley rats were subjected to SNL and SNI according to previously described methods. In both models, the rats were allowed to recover from the surgery and were assessed for tactile and thermal allodynia symptoms over an 8-week follow-up period. Morphine, pregabaline and amitriptyline were dosed and tested for their efficacy to alleviate the symptoms in the end of chronic follow-up period. During the follow-up period, fine motor gait analysis was performed on the models to understand how spontaneous gait properties are affected in the models and what additional information they provide from the model besides classical stimulated sensory responses. In both of the models, SNL and SNI, models showed clear tactile allodynia. Thermal responses were found to be either transient or more variable than tactile allodynia seen in the model. Pharmacological treatments with morphine, pregabaline and amitriptyline showed expected reversal of the tactile allodynia. Results from the thermal allodynia were more variable in tests, both in the magnitude of thermal allodynia over time as well as responses to pharmacological treatments. When both models were assessed in fine motor gait analysis, we saw clearly altered gait parameters in rats subjected to SNL and SNI. These data provide more in depth insight to neuropathic pain models. Moreover, the use of gait as an endpoint provides a functional endpoint that significantly increases the utility of the model in its application to research and drug development.

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Poster

063. Pain Models: Behavior I

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Topic: D.08. Pain

Support: Texas Norman Hackeman Advanced Research Program (003656-0071-2009)

TxMRC Grant

Title: A novel model of spontaneous pain behaviors in rats generated by controllable electrical stimulation of the peripheral nerve

Authors: ***A. HARRIS**[1,2], A.-L. LI[3], M. M. KAJUMBA[2], J. N. STRAND[2], P. N. FUCHS[2], Y. B. PENG[2];
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Abstract: Neuropathic pain patients have described experiencing spontaneous pain attacks with shooting, stabbing, and burning qualities (Campbell & Meyer, 2006). Rodent models are either acute (inflammation by formalin) or cannot be manipulated *in vivo* by the experimenter during an experiment (nerve injury). We propose a novel model of spontaneous pain in rodents that can be manipulated throughout the course of an experiment; the cuff stimulating electrode model. Two soft implantable wires were unilaterally implanted at the L5 spinal nerve location in 15 Sprague-Dawley rats. Seven animals were excluded due to nerve damage. After recovery, our custom-built wireless stimulating device (Zuo et al, 2012) was attached to the wires to deliver variable stimulations to the nerve in freely moving animals. A repeated measures ANOVA of data from the mechanical paw withdrawal threshold test of hypersensitivity confirmed that there was no hypersensitivity created by the surgical implant. A test of spontaneous pain behaviors was applied using the formalin scoring method (up, down, & lick) during stimulation. Stimulations were applied for 10 seconds with 1 minute of rest over 10 trials. Higher stimulation parameters yielded a significant increase in spontaneous pain behaviors ($p<.05$) while low parameters did not. Even further, high parameter stimulation resulted in spontaneous pain behaviors that persisted into the 5 minute resting period when no stimulation was applied. A modified Place Escape Avoidance Paradigm was used to measure the unpleasantness of stimulation. Stimulations were administered every 15 seconds for a 30 minute period: high electrical stimulations (100Hz, 1V, 10sec) were delivered while animals were on the dark, naturally preferred half of a testing chamber and low stimulations (50 Hz, .5V, 10sec) were applied while animals were on the light side. Repeated measures ANOVA indicated that while animals did not spend increasingly more time choosing to receive lower stimulation, the average time spent on the light side of the chamber was consistent with previously published results for animals with a pain model. We conclude that our model can be used to study spontaneous pain behaviors in freely moving rodents when stimulation is administered at the high parameter (100 Hz, 1V). Benefits of the model include: (1) the ability of the experimenter to manipulate spontaneous input to investigate spontaneous pain behaviors (2) reduce the number of animal controls that are needed because varying degrees of pain can be studied within the same animal and (3) to investigate the complex relationship between peripheral input and brain activity to create dynamic response profiles.

Disclosures: **A. Harris:** None. **A. Li:** None. **M.M. Kajumba:** None. **J.N. Strand:** None. **P.N. Fuchs:** None. **Y.B. Peng:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.23/M10

Topic: D.08. Pain

Support: NeuroDigm Co.

Title: NeuroDigm GEL(TM) model of neuropathic pain in rats: a nonsurgical model with an analgesic profile as seen in man

Authors: *D. A. FITTS[1], J. L. BRYANT[2], M. R. HANNAMAN[3];
[1]Univ. Of Washington (retired), Arlington, WA; [2]Inst. of Human Virology, Univ. of Maryland, College Park, MD; [3]NeuroDigm Corp., Colorado Springs, CO

Abstract: A percutaneous placement of a physiologic gel near a peripheral nerve gradually induces known neuropathic pain behaviors. Rats were given gel injections in the tibial nerve tunnel on the left (ipsilateral) lower leg (GEL group, n = 14), injections of physiologic Ringer's Lactate Ph 7.4 on the same side (Sham group, n = 8) or no injection (Control group, n = 11). Paw-withdrawal responses to application of 3 von Frey fibers, a sable brush, and pinprick were tested on both hind paws on 4 pretreatment baseline days and periodically on 36 days between the treatment day 0 and post-treatment day 149. An increase in paw withdrawals to the chosen stimuli is considered neuropathic pain behavior. There were 3 time periods of screening in the 5 months with the same 4 analgesics: morphine sulfate 3 mg/kg, celecoxib 10 mg/kg, gabapentin 25 mg/kg, or duloxetine 10 mg/kg. Static (von Frey fibers) and dynamic (brush) mechanical allodynia and mechanical hyperalgesia (pinprick) emerged in the GEL group after 23 days on the ipsilateral side and to a lesser extent and after 72 days on the contralateral side. The paw-withdrawal response to pinprick was robust, occurred in every Gel-injected animal, and persisted throughout the 149 days. Morphine was effective in reducing paw withdrawal responses early but not late in the post-treatment period. Celecoxib was ineffective. Gabapentin and duloxetine were both effective throughout the post treatment period. The pattern of development of ipsilateral and contralateral symptoms, the persistence of symptoms for months, and the analgesic profiles of response and dosage closely model human neuropathic pain.

Disclosures: **D.A. Fitts:** 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; NeuroDigm Co.. **J.L. Bryant:** None. **M.R. Hannaman:** E. Ownership Interest (stock, stock options, royalty,

receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroDigm Corp..

Poster

063. Pain Models: Behavior I

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Support: IMI EUROPAIN grant nr 115007

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EFPIA companies in kind

Ulla & Gustaf af Uggles Foundation

KI Foundation

GLORIA (FP7) European Research Council

Title: Prospective multi-center validation of a burrowing paradigm as an ethologically-relevant readout in a rat model of acute inflammation-evoked pain

Authors: *K. RUTTEN[1], R. WODARSKI[2], A. DELANEY[3], C. ULTENIUS[3], R. MORLAND[2], A. LINDSTEN[4], .-. EUROPAIN BURROWING STUDY GROUP[5], A. S. C. RICE[2]; [1]Grünenthal, GmbH, Aachen, Germany; [2]Imperial Col. London, London, United Kingdom; [3]Karolinska Institutet, Solna, Sweden; [4]Lundbeck A/S, Valby, Denmark; [5]Multiple Res. Centers:, detailed on poster, Germany

Abstract: In this prospective multi-center validation study the burrowing paradigm was validated across 8 centers in Europe, USA and Japan using a standardized protocol in a Complete Freund's Adjuvant model (CFA) for inflammatory pain. Burrowing is an ethologically relevant rodent behavior, suppressed by pain and other conditions, and considered an important non-evoked readout for ongoing pain. Methods: Participating labs followed a standardized burrowing protocol designed according to ARRIVE Guidelines. Burrowing performance was measured by the weight of substrate left in a hollow tube after 60 minutes exposure. Male rats were given daily burrowing sessions for 2-4 days in pairs after which individual baseline burrowing scores were taken (2-3 days). Following baseline, animals where randomly allocated to one of three

groups: Naïve (untreated), Sham (vehicle -100 µl 0.9% saline i. pl.), or CFA (100 µg (100 µl @ [1 mg/ml]) i. pl.). Burrowing was tested on post treatment days 1, 2, 3, 7 and 10. Data were centrally captured, monitored and checked, including minor protocol variations permitted for local expediency. Data were analyzed by a restricted maximum likelihood (REML)-based mixed model for repeated measures (MMRM) approach. Results: 11 studies at 8 different laboratories were included, with a total of 104 CFA treated rats, 96 sham treated and 49 naïve. The analyses of change from baseline over time in burrowing score provided a consistent picture (including and excluding Lab ID as explanatory factor). In all analyses there was a significant difference between CFA and control (sham or naïve) in change in burrowing score at day 1 after injection and the CFA difference decreased over time. Lab ID was statistically significant when included in the model, but when adjusted for Lab ID, the estimates of change from baseline showed similar patterns in both analyses, suggesting the burrowing effect seen in the CFA model is robust across laboratories. The baseline score-by-time interaction was not significant, while all other fixed effects contributed statistically significantly. In addition, burrowing scores have been investigated for the most important explanatory factors based on descriptive statistics, as no statistical hypothesis testing was possible due to study design. Conclusion: A robust and positive replication of the CFA effect on burrowing behaviour across centres was observed and the timescale of effects is compatible with what is known of biology of CFA effects. In preclinical settings this multi-center validation approach is unique, in contrast to the usual ad-hoc replication which is haphazard and subject to publication and single center bias.

Disclosures: **K. Rutten:** A. Employment/Salary (full or part-time):; Grünenthal, GmbH. **R. Wodarski:** None. **A. Delaney:** None. **C. Ultenius:** None. **R. Morland:** None. **A. Lindsten:** None. **-. EUROPAIN Burrowing study group:** None. **A.S.C. Rice:** None.

Poster

063. Pain Models: Behavior I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.25/M12

Topic: D.08. Pain

Support: NIH/NINDS NS072204

Title: Meningeal application of low pH, IL-6 and allyl isothiocyanate produces migraine-related behavior in mice

Authors: ***C. C. BURGOS-VEGA**[1], G. TREVISAN[3], L. QUIGLEY[2], T. PRICE[2], G. DUSSOR[2];

[2]Brain & Behavior Sci., [1]UT DALLAS, Dallas, TX; [3]Federal Univ. of Santa Maria, Santa Maria, Brazil

Abstract: Migraine headache is the most prevalent neurological disorder but the pathophysiology contributing to the development and progression of the condition is not well understood. Current pharmacological treatments are inadequate at managing the complex symptomology experienced by migraine sufferers and thus new therapeutics are needed. Increasing amounts of evidence suggest that nociceptor activation in the meninges plays a role in migraine pain. Identification of mechanisms leading to activation/sensitization of these neurons may provide targets for novel migraine therapeutics. Previous work in rat models of migraine pain has found that stimulation of the dura produces afferent input to the central nervous system leading to headache-like behaviors (cutaneous allodynia). However, development of a mouse model of headache using dural stimulation would allow the use of genetic tools for target identification that do not exist in rats. The intent of these studies was to determine whether exposure of mouse dura to stimuli that have been shown to produce headache behaviors in rats (low pH, IL-6, and 10% Allyl isothiocyanate (AITC)) produce similar responses in mice. Male ICR mice were acclimated to testing chambers for 2-3 days for approximately 2 hours a day. Prior to injection, baseline facial and hindpaw thresholds were recorded for each animal. The junction of the saggital and lambdoidal sutures was identified and this junction was used for injection of solutions onto the dura using a 0.5 mm injector designed to penetrate the skull but not damage the dura. The mice were under light isofluorane anesthesia for less than 2 minutes and injections of 5 µl were administered onto the dura. The animals were then returned to the testing boxes. Following administration, facial and hindpaw thresholds were measured at 1,3, 5 and 24 hours post-injection using the Von Frey up down method of testing. Dural application of pH 6.0, IL-6, and 10% AITC produced facial and hindpaw allodynia, lasting up to 24 hours, that was not present in animals given vehicle injections. These data show that stimulation of dural afferents in mice with pH 6.0, IL-6, and AITC produce headache-like behaviors similar to those previously demonstrated in rats. Further, they suggest that mice are a suitable species for behavioral testing of headache following stimulation of the dura mater.

Disclosures: C.C. Burgos-Vega: None. **G. Trevisan:** None. **L. Quigley:** None. **T. Price:** None. **G. Dussor:** None.

Poster

063. Pain Models: Behavior I

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 63.26/M13

Topic: D.08. Pain

Support: NIH Grant NS085510

Title: EEG analysis of a rodent headache model

Authors: A. MELO-CARRILLO[1], R. NOSEDA[1], F. J. FLORES[2], R. BURSTEIN[1], *A. M. STRASSMAN[1];
[1]Dept. Anesthesia, Beth Israel Deaconess Med. Ctr., Boston, MA; [2]MIT, Cambridge, MA

Abstract: 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). In the present study, we used EEG recording in this model to investigate whether the resting behavior of the stimulated animals differed from that of the control animals, despite being indistinguishable with video analysis. We hypothesized that, if the resting behavior of the stimulated animals represents a behavioral correlate of headache, then it constitutes a different behavioral state than the resting behavior of control animals. Specifically, we hypothesized that the EEG would lack the increased slow wave activity that would be expected during normal resting and transition to sleep, and would instead display signs of arousal that do not normally accompany periods of behavioral quiescence in control animals. Following a 5-day pre-surgery habituation period, male Sprague-Dawley 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. As observed previously, animals that received inflammatory mediators displayed more resting behavior than control animals. In both stimulated and control animals, the resting periods were accompanied by an increase in delta activity compared to the intervening periods of exploratory behavior, and this delta activity tended to increase over the course of each individual rest period. Preliminary analysis indicates that the resting behavior in the stimulated animals was accompanied by a similar level of slow wave activity, but an increased amount of activity in a band extending from the upper delta to the theta range, as compared to resting behavior in the control animals. Such a difference would be consistent with findings of EEG studies in human pain patients, and would support the hypothesis that the periods of behavioral quiescence in the stimulated animals represent a different state than the resting periods exhibited by control animals.

Disclosures: **A. Melo-Carrillo:** None. **R. Noseda:** None. **F.J. Flores:** None. **R. Burstein:** None. **A.M. Strassman:** None.

Poster

063. Pain Models: Behavior I

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Topic: D.08. Pain

Support: Buoniconti Fund

UM Office of Research

Research Education

Innovative Medicine (RIM) Research Support Award

Title: Development of a conditioned place preference model to evaluate post-TBI headache in rats

Authors: *J. SAGEN[1], S. JERGOVA[1], D. HOPMAN[1,3], J. MATOS[2], O. FURONES-ALONSO[1], H. BRAMLETT[1], S. IZENWASSER[2];
[1]Miami Project Cure Paralysis, [2]Dept. of Psychiatry & Behavioral Sci., Univ. Miami,Sch Med., Miami, FL; [3]Avans Univ., Breda, Netherlands

Abstract: Headache pain is a common symptom following traumatic brain injury (TBI), with a significant patient population continuing to experience recurring or persistent headaches for years. Although widely accepted rodent models for TBI exist, they have not been utilized in evaluation of headache pain or exploration of effective treatment strategies. Therefore the goal of this study was to establish a predictive rat model for evaluating post-TBI headache. A novel conditioned place preference (CPP) model was established to reflect headache pain as experienced by the patient, utilizing common headache triggers such as bright light. Behavioral manifestation of headache like symptoms in the form of facial allodynia also was evaluated. The fluid percussion injury model is widely accepted as a clinically relevant model for TBI in that it produces cognitive and motor deficits consistent with TBI injury severity and clinical observations. The right parietal cortex was partially exposed by craniotomy in male rats and a fluid percussion device was used to induce moderate TBI. Sham rats underwent the same procedures omitting the injury. Two weeks post TBI/sham surgery animals were evaluated for the presence of headache-like behavior by a CPP setup. Cages with white walls on one side and striped black and white walls on the other side were used with a bright light over the white side of the cage. The time spent on each side was measured over a 20 minute period (pretest). Next a divider was inserted in the middle of the cage and animals were conditioned for the dark side

with no food, and the light side with food. After 5 days of conditioning, the divider was removed and the same conditions as during the pretest were used to evaluate time spent on each side (posttest). Sham animals spent significantly more time in the white side of the cage with bright light on during the posttest as compared to pretest and compared to TBI animals, indicating development of CPP. TBI rats preferred to stay in the dark side. However, when the posttest was run with the light off, TBI rats showed a preference for the side originally paired with food, suggesting that the light was aversive. Pre-treatment with sumatriptan, a drug used clinically for treatment of migraine headaches, reversed bright light avoidance in TBI animals. Facial allodynia was evaluated in animals after 30 min of exposure to light. Most of the TBI animals that developed light aversion also were sensitive to nonnoxious stimulation with Von Frey hairs in the periocular and forehead region. These results suggest that TBI may induce headache-like behavior and that our model of CPP may be suitable for future studies of experimental treatment of post-TBI headache.

Disclosures: J. Sagen: None. **S. Jergova:** None. **D. Hopman:** None. **J. Matos:** None. **O. Furones-Alonso:** None. **H. Bramlett:** None. **S. Izenwasser:** None.

Poster

064. Pain Models: Behavior II

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Topic: D.08. Pain

Support: CIHR

QPRN

NSERC

LAEF

Title: Optogenetic investigation of the MRGPRD nociceptors in behaving transgenic mice

Authors: *I. DAOU, A. R. ASE, P. SEGUELA;
Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada

Abstract: Primary afferents consist of a heterogeneous population of peripheral neurons. Nociceptors represent a subset of sensory neurons responsible for detecting and transmitting pain signals from the periphery to the central nervous system. The molecular profiling of nociceptors

classifies them into smaller subpopulations associated with a variety of sensory functions and modalities. The Mas-related G protein-coupled receptor D (MRGPRD) is expressed in a population of nociceptive non-peptidergic polymodal C-fibers that largely overlaps with fibers expressing the purinoceptor P2X3. MRGPRD neurons are implicated in the detection of mechanical pain under normal and inflammatory conditions. The β-alanine-responsive subset of these neurons is also heat-sensitive and produces itch in response to β-alanine. Using the MRGPRD-CreERT2 mouse line (Wenqin Luo, U. Penn), we selectively delivered the excitatory opsin ChR2-EYFP to adult MRGPRD neurons by inducing its expression postnatally to overcome any developmental regulation of MRGPRD. This optogenetic approach allows us to manipulate this neuronal population with high spatio-temporal precision. EYFP fluorescence revealed efficient and selective expression of the transgenic opsin in dorsal root ganglia (DRG), trigeminal ganglia, sciatic nerve, glabrous skin and dorsal horn of the spinal cord. EYFP-labeled projections to the dorsal horn targeted lamina II, consistent with previous reports showing monosynaptic input from IB4-labeled MRGPRD+ afferents to second-order neurons in lamina II. Electrophysiological recordings on cultured DRG neurons showed blue light-evoked inward photocurrents with typical ChR2 kinetics, leading to reliable generation of action potentials. EYFP-expressing DRG neurons were also responsive to the P2X3 agonist αβmeATP, confirming the specific expression of ChR2 in non-peptidergic neurons. *In vitro* validation data translated *in vivo* into nocifensive behaviors as transdermal stimulation with acute blue light evoked hind paw withdrawal and licking. Further evaluation of this model is underway to investigate the role of the MRGPRD neuronal population in different sensory modalities and under chronic pain conditions. This work will contribute to expanding our knowledge on the functional diversity of somatosensory pathways and their involvement in nociceptive transduction and pain processing.

Disclosures: **I. Daou:** None. **A.R. Ase:** None. **P. Seguela:** None.

Poster

064. Pain Models: Behavior II

Location: Hall A

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Topic: D.08. Pain

Support: NIH Grant TR01 NS081707

Title: Optogenetic inhibition of specific populations of sensory neurons mediating bladder nociception

Authors: *A. D. MICKLE[1], V. K. SAMINENI[2], S. PARK[3], M. PULLEN[2], J. GRAJALES-REYES[2], C. D. MORGEN[1], J. RODGERS[3], R. W. GEREAU, IV[2];
[1]Washington Univ., Saint Louis, MO; [2]Washington Univ. Pain Ctr., Saint Louis, MO; [3]Univ. of Illinois, Urbana-Champaign, IL

Abstract: Bladder pain is a cardinal sign of interstitial cystitis/bladder pain syndrome (IC/BPS); however, its pathophysiology is poorly understood. A greater understanding of the neuronal populations underlying IC/BPS would be greatly beneficial in the development of new treatments. Here, we used an optogenetic strategy to inhibit different populations of bladder afferents and characterize the influence that inhibition has on bladder nociception. We characterized two transgenic mouse lines expressing the light-activated proton pump, Archaerhodopsin (Arch), fused to EYFP. One line expressed Arch in Advillin+ neurons (all sensory neurons) and the other expresses Arch in Nav1.8+ neurons (nociceptors). Histological studies confirmed the expression of Arch-EYFP in the bladder-projecting sensory neurons. Electrophysiological analysis of sensory neurons from these mouse lines confirmed robust light-induced inhibition of action potential firing in cells expressing Arch-EYFP. To study the impact of optogenetic inactivation of these neuronal populations on bladder nociception, we assessed the visceromotor response (VMR) during graded bladder distension (20-60 mmHg), both prior to and during 532nm green laser stimulation of the bladder. In our preliminary studies, activation of Arch in both Advillin+ and Nav1.8+ bladder neuron populations decrease the visceromotor response (VMR) during graded bladder distension, suggesting activation of Arch in the subpopulation of Nav1.8+ expressing neurons causes a decrease in bladder sensitivity to nociceptive stimulation. Further studies utilizing this approach will help to elucidate the cellular basis of bladder pain in chronic bladder pain syndromes. This work is supported by an NIH Director's Transformative Research Award (TR01 NS081707) to RG and JR.

Disclosures: A.D. Mickle: None. **V.K. Samineni:** None. **S. Park:** None. **M. Pullen:** None. **J. Grajales-Reyes:** None. **C.D. Morgen:** None. **J. Rodgers:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder of a company working to develop the technology. **R.W. Gereau:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder of a company working to develop the technology.

Poster

064. Pain Models: Behavior II

Location: Hall A

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Topic: D.08. Pain

Title: Optogenetic inhibition of the corticalstriatal circuit intensifies sensory and affective symptoms of chronic neuropathic pain

Authors: *R. YANG, H. LIN, J. WANG, C. SU, T. R. MANDERS;
Anesthesiol., NYU Med. Sch., New York, NY

Abstract: Cortical mechanisms that regulate acute or chronic pain remain poorly understood. Optogenetic activation of the prefrontal cortex (PFC) has been shown to alleviate both sensory and affective effects of chronic pain (Lee et al. 2015). Furthermore, projections from the PFC to the nucleus accumbens (NAc) have been shown to mediate these pain-relieving effects. Thus, this corticostriatal circuit plays an important pain modulatory role. However, it is unclear whether this circuit is endogenously involved in pain regulation. To answer this question, we disrupted this circuit using an optogenetic strategy. We used halorhodopsin, a light activated chloride channel, to optogenetically inhibit the PFC and then selectively inhibit the axonal projection from the PFC to the NAc in rats that have chronic neuropathic pain. Our results reveal that inhibition of the PFC or its projection to the NAc heightens both sensory and affective symptoms pain. Thus, these results suggest that the corticostriatal circuit likely plays an important role in endogenous pain regulation.

Disclosures: **R. Yang:** None. **H. Lin:** None. **J. Wang:** None. **C. Su:** None. **T.R. Manders:** None.

Poster

064. Pain Models: Behavior II

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 64.04/M18

Topic: D.08. Pain

Title: A mouse model of oxaliplatin-induced neuropathic pain

Authors: *T. T. AHTONIEMI, A.-M. ZAINANA, L. KOISTINEN, R. HODGSON, A. NURMI;
Charles River Discovery Res. Services, Kuopio, Finland

Abstract: Neuropathic pain is globally a significant factor resulting in decreased quality of life. Causes as well as symptoms of neuropathic pain are diverse ranging from numbness to tingling, touch sensitivity and sensitivity to cold or warm temperatures. As many of the chemotherapeutic drugs such as platin-based compounds are also neurotoxic, they are often associated with neuropathic pain during and after therapy. To model platin-based chemotherapy induced neuropathic pain in rodents we established and validated a sustained chronic model of oxaliplatin-induced neuropathic pain in mice which suffer from cool/cold allodynia. Mice were treated withoxaliplatin (3 mg/kg; i.v.) or vehicle every 3 days for 27 days. After a stabilization period of one week after discontinuation of the oxaliplatin the mice were tested weekly for their sensitivity for cool/cold allodynia until 110 days after the induction of the model. A tail flick test was performed by submerging the tail in +15°C water and measuring the flick response latency. An acetone test was performed during the follow-up period by measuring response (flicking, shaking and licking) duration when cool stimulus (acetone drop) was placed on dorsal side of the hind paw. Pregabaline, an antiepileptic drug and duloxetine, a selective noradrenaline reuptake inhibitor (SNRI), were tested for their ability to alleviate the cool allodynia symptoms. At the baseline, mice showed normal responses to cool/cold stimuli. Oxaliplatin challenge induced both acute and chronic cool allodynia symptoms during and after the induction phase. Cool allodynia was seen in tail flick and acetone tests and was persistent until 110 days. Pregabaline (20, 40 and 80 mg/kg) and duloxetine (1, 3 and 10 mg/kg) were effective in the acetone test, but in the tail flick test results were more variable or not significantly analgesic. These data demonstrate the utility of oxaliplatin treatment as a model of chemotherapy-induced neuropathic pain with the presentation of cool/cold allodynia symptoms. Moreover, the duloxetine and pregabaline demonstrate the utility of this model for the development of novel treatments.

Disclosures: **T.T. Ahtoniemi:** None. **A. Zainana:** None. **L. Koistinen:** None. **R. Hodgson:** None. **A. Nurmi:** None.

Poster

064. Pain Models: Behavior II

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Title: Short-term sleep disturbance-induced stress does not affect basal pain perception, but does delay postsurgical pain recovery

Authors: *Y. TAO, P.-K. WANG, J. CAO, L. LIANG, B. M. LUTZ, A. BEKKER;
Dept. of Anesthesiol., Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Chronic sleep disturbance-induced stress is known to increase basal pain sensitivity. However, most surgical patients frequently report short-term sleep disturbance/deprivation during pre- and post-operation periods and have normal pain perception pre-surgery. Whether this short-term sleep disturbance affects postsurgical pain is elusive. We here reported that pre- or post-exposure to rapid eye movement sleep disturbance (REMSD) 6 h daily for 3 consecutive days did not alter basal responses to mechanical, heat, and cold stimuli, but did delay recovery in incision-induced reductions in paw withdrawal threshold to mechanical stimulation and paw withdrawal latencies to heat and cold stimuli on the ipsilateral side of male or female rats. This short-term REMSD led to stress evidenced by an increase in swim immobility time, a decrease in sucrose consumption, and an elevation in the level of corticosterone in serum. Blocking this stress via intrathecal RU38486 or bilateral adrenalectomy abolished REMSD-caused delay in recovery of incision-induced reductions in behavioral responses to mechanical, heat, and cold stimuli.

Disclosures: Y. Tao: None. **P. Wang:** None. **J. Cao:** None. **L. Liang:** None. **B.M. Lutz:** None. **A. Bekker:** None.

Poster

064. Pain Models: Behavior II

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Rita Allen Foundation

Title: Endothelin-type A receptors are necessary for pain hypersensitivity in a mouse model of Sickle Cell Disease

Authors: *B. LUTZ, Y.-X. TAO;
Anesthesiol., Rutgers New Jersey Med. Sch., Newark, NJ

Abstract: Sickle Cell Disease (SCD), a hemoglobinopathy resulting in a mutated β globin gene, is associated with acute painful episodes and persistent intractable pain. Both adults and children report chronic pain symptoms that are severe enough to require long-term opioid therapy. Endothelin-1, a known pain inducer, is elevated in the blood plasma of both SCD patients and SCD mouse models. Endothelin-1 binds to Endothelin-type A receptors and Endothelin-type B receptors. In dorsal root ganglion (DRG), ETA receptors are found in the neurons, while ETB receptors are found in the surrounding satellite cells. We hypothesize that ET-1 binding to ETA receptors on peripheral nerve terminals initiates nociceptor sensitization which contributes to chronic pain in a mouse model of SCD. Mechanical, thermal, and cold sensitivity were assessed in 6 months old HBSS-BERK (SCD) and HBAA control mice before and after exposure to hypoxia with and without the addition of the ETA receptor antagonist, ABT-627. Additionally, a DRG-specific genetic knockout of ETA receptors underwent total body irradiation and bone marrow transplantation (BMT) using HBSS and HBAA bone marrow. The resulting ETA receptor knockout mice expressed human sickle β globin (HBSS marrow recipients) or normal human β globin (HBAA marrow recipients). Pain behavior was assessed in these mice before BMT, after BMT, and after hypoxia. Our results show that HBSS mice possess basal pain hypersensitivity that is exacerbated after 3 hours of exposure to a hypoxic environment. Subcutaneous injection of ABT-627 attenuated basal and hypoxia-exacerbated pain hypersensitivity in HBSS mice. Additionally, DRG ETA receptor knockout mice transplanted with HBSS bone marrow showed less basal and hypoxia-induced pain hypersensitivity compared to ETA $^{flox/flox}$ mice transplanted with HBAA bone marrow. Our findings indicate that ETA receptors are necessary for pain hypersensitivity observed in SCD mice.

Disclosures: B. Lutz: None. **Y. Tao:** None.

Poster

064. Pain Models: Behavior II

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 64.07/M21

Topic: D.08. Pain

Support: NIH Grant DA027625

Title: Continuous home cage monitoring of wheel-running in male and female rats: A clinically relevant method to assess nociception

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Abstract: Failures in analgesic drug development have raised concern over the translatability of nociceptive research in animals. Part of the problem is that a decrease in activity is often used to evaluate pain in patients, whereas nociception in animal studies is commonly assessed with acute noxious stimuli such as the hot plate and tail flick tests. Such pain-evoked tests do not mimic the disruptive effects of pain on daily life, thus pain-depressed tests have been developed to address this issue. The present study expands on this approach by examining depression of wheel-running. Wheel-running is a natural rodent behavior that can be assessed in the rat's home cage (a low-stress environment) 24 hours a day. If depression of wheel-running mimics the effects of pain in humans, then the induction of inflammatory pain should depress wheel-running, and this reduction should be greater in female compared to male rats (human data reveal a greater prevalence of chronic pain in women). Male and female rats were group housed by sex until at least age 50 days prior to the experiment. Rats were transferred to individual cages with a running wheel where they remained for the rest of the experiment. Rats were allowed free access to the wheel 23 hours/day for either 3 or 8 days prior to the induction of hindpaw inflammation. Wheel-running during the 23 hours immediately prior to induction of inflammation was used as the baseline. Inflammation was induced with an intraplantar injection of Complete Freund's Adjuvant (CFA; 0.1 mL) into the right hindpaw. Rats were removed from the home cage for one hour each day to assess nociception using mechanical and thermal pain-evoked tests (von Frey and Hargreaves, respectively). Baseline wheel-running was greater in female (2609 revolutions) compared to male rats (824 revolutions), and following 8 compared to 3 days of prior exposure to the wheel (2268 vs. 1627 revolutions). CFA almost completely inhibited wheel-running in male and female rats in the 23 hours following administration. Wheel-running recovered to near baseline levels by Day 3 in male rats, whereas recovery of wheel-running took 4 days in female rats. Withdrawal thresholds for the mechanical and thermal evoked tests were low across the entire 4 days of testing. These data demonstrate that depression of wheel-running mimics the effects of pain in humans in two important ways: Induction of inflammatory pain reduces daily activity, and this depression of behavior is enhanced in females compared to males. Taken together, our results suggest that continuous monitoring of wheel-running activity provides a novel, simple, and reliable way to assess nociception in male and female rats.

Disclosures: R. Kandasamy: None. **J.J. Calsbeek:** None. **M.M. Morgan:** None.

Poster

064. Pain Models: Behavior II

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Topic: D.08. Pain

Support: NS072206

HL117684

DA033390

Title: Short-term perioperative stress does not alter basal pain perception, but does exacerbate postsurgical pain

Authors: *J. CAO[1], P.-K. WANG[2], V. TIWARI[4], L. LIANG[3], B. M. LUTZ[3], W.-D. ZANG[5], A. BEKKER[3], X.-Q. GAO[5], Y.-X. TAO[1];
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Abstract: It has been reported that chronic stress increases basal pain sensitivity and/or exacerbates existing persistent pain. Nevertheless, the majority of surgical patients are under normal physiological and psychological conditions and have normal pain perception before surgery although they have short-term stress during perioperative period. Whether or not this short-term stress affects persistent postsurgical pain is unclear. We reported here that pre- or post-exposure to immobilization or forced swimming 6 h daily for 3 consecutive days did not change basal responses to mechanical, heat, and cold stimuli, but did exacerbate postsurgical pain evidenced by delaying recovery in incision-induced reductions in pain withdrawal threshold to mechanical stimulation and paw withdrawal latencies to heat and cold stimuli on the ipsilateral side of male or female rats. This short-term immobilization produced stress demonstrated by an increase in swim immobility time, a decrease in sucrose consumption, and an elevation in the level of corticosterone in serum. Attenuating this stress via intrathecal a selective glucocorticoid receptor antagonist RU38486 or bilateral adrenalectomy abolished stress-caused delay in recovery of incision-induced reductions in behavioral responses to mechanical, heat, and cold stimuli. Our results indicate that short-term stress during perioperative period worsens postoperative pain although it does not affect basal pain perception. Prevention of short-term stress may help the recovery of postoperative pain in patients.

Disclosures: **J. Cao:** None. **P. Wang:** None. **V. Tiwari:** None. **L. Liang:** None. **B.M. Lutz:** None. **W. Zang:** None. **A. Bekker:** None. **X. Gao:** None. **Y. Tao:** None.

Poster

064. Pain Models: Behavior II

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Support: NIH NCCIH IRP funding

Title: Intense and mild stress can cause stress-induced analgesia on the formalin pain test two weeks after MRI restraint training

Authors: *L. A. LOW, L. C. BAUER, I. RAUF, L. HYSON, C. BUSHNELL;
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Abstract: Aim Functional MR imaging of awake rodents is beginning to be utilized in pain research, but the training involves repeated physical restraint and can be stressful, potentially causing lasting effects on behavior and brain function. In this study, we replicated previously published fMRI restraint training protocols and investigated responses to the formalin pain test 12 days later. Methods: Male Long Evans rats (n=12 per group) were subject to one of the following treatments for 30 minutes per day on 3 consecutive days: 1) physical restraint plus loud (~90dB) MR scanner noise (R); 2) exposure to restraint equipment plus quiet (15dB) scanner noise, performed in the same testing room as rats were previously restrained (NR); or 3) no restraint, no scanner noise (Control, C). On the third day, a noxious heat stimulus was applied via thermode to the left hind paw of R and NR rats (4 x 48°C for 32s, 36s ISI). Blood samples were taken at baseline, every day after restraint, and 24 hours post-restraint, and plasma corticosterone levels quantified by ELISA. Twelve days after the final restraint/handling session rats received 1% formalin (50µl) s.c. injection to the hindpaw and behavior was observed for 60 minutes. Pain behavior was quantified by calculation of a weighted pain score. Results: Restrained rats showed the highest corticosterone response after restraint compared to non-restrained and control animals (R = 189% of baseline, NR = 146% of baseline, C = 125% of baseline, $p<0.0001$). All animals showed an increase in stress hormone after brief restraint and thermode application on day 3 (R = 256% of baseline, NR = 206% of baseline, C = 171% of baseline, $p<0.0001$). When subject to the formalin pain test 12 days after the final restraint, both R and NR groups showed significantly lower weighted pain scores on the formalin test compared to controls (R= 0.23, NR = 0.23, C = 0.47; $p<0.0001$). Conclusions: Restraint causes increases in stress hormones. However, an increase is also seen in non-restrained animals exposed to olfactory cues from stressed animals. An acute nociceptive stimulus causes increases in stress hormones in all animals. Two weeks after restraint, previously restrained and non-restrained animals both show similar reductions in pain behaviors on the formalin test relative to controls,

suggesting a long-lasting form of stress-induced analgesia. Researchers studying nociception should be aware that both intense and mild stressors can have pronounced and lasting effects on pain behaviors.

Disclosures: L.A. Low: None. **L.C. Bauer:** None. **I. Rauf:** None. **L. Hyson:** None. **C. Bushnell:** None.

Poster

064. Pain Models: Behavior II

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Topic: D.08. Pain

Support: Project SIP 20150031

Project CONACyT 178027

Arroyo Lira AG is a CONACyT fellow (Grant Number 269377)

Title: Evaluation of the antinociceptive and anti-inflammatory effect and gastric safety of the combination of docosahexaenoic acid and naproxen in rats

Authors: *A. G. ARROYO LIRA, E. A. PINEDA PEÑA, A. E. CHÁVEZ PIÑA; ESCUELA NACIONAL DE MEDICINA Y Homeopatía DEL INST, Mexico, Mexico

Abstract: Naproxen is a traditional nonselective nonsteroidal anti-inflammatory drug (NSAID) prescribed in the management of pain and inflammatory diseases like arthritis. Nonetheless, like other traditional nonselective NSAIDs cause gastric damage and other side effects. In other hand, the use of combination of analgesics is a common practice intended to achieve one or more therapeutic goals; for example improving efficacy without increasing side effects. The aim of this work was to evaluate the antinociceptive and anti-inflammatory effect of oral administration of naproxen when is administered individually or in combination with docosahexaenoic acid (DHA), and define the gastric safety and pharmacodynamic interaction in the combination. Female Wistar rats were orally administered naproxen (10, 30, 100 and 300 mg/kg), DHA (56.23, 100, 177.83 and 316.23 mg/kg) the antinociceptive effect was evaluated with the formalin test and the anti-inflammatory effect was evaluated inducing the paw edema by carrageenan. Effective dose 30 (ED30) for naproxen and DHA was determined for their antinociceptive and anti-inflammatory effect, then the dose response curve for the combination of compounds in a fixed-dose ratio 1:3 was performed for the antinociceptive effect (6.80, 13.61,

27.21 and 54.42 mg/kg). Also for the anti-inflammatory effect the dose response curve for combination in a fixed-dose ratio 1:3 was performed (4.48, 8.96, 17.93 and 35.85 mg/kg). An isobolographic analysis was performed to characterize the pharmacodynamic interaction between naproxen and DHA. Gastric hemorrhagic lesions were measured for each group of rats. The antinociceptive ED30 values were 27.67±3.80 mg/kg, 134.83±27.72 mg/kg and 33.44±1.90 mg/kg p.o., for naproxen, DHA and the combination respectively; while theoretical ED30 of the combination was 54.42±7.49 mg/kg. Naproxen produced 30.91±5.73 mm2 of gastric damage, while the combination did not induce gastric damage. For the inflammatory effect, the ED30 values were 5.18±1.42 mg/kg, 127.88±12.47 mg/kg, and 12.33±4.61mg/kg p.o. respectively; the value of the theoretical ED30 of the combination was 35.86±3.29 mg/kg. The isobolographic analysis of the theoretical and experimental ED30 of the combination shows a synergistic interaction in the antinociceptive and anti-inflammatory effect. In conclusion, these data suggest that oral administration of naproxen-DHA combination induces a synergistic antinociceptive and anti-inflammatory effect and gastric safety.

Disclosures: **A.G. Arroyo lira:** None. **E.A. Pineda Peña:** None. **A.E. Chávez Piña:** None.

Poster

064. Pain Models: Behavior II

Location: Hall A

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Program#/Poster#: 64.11/M25

Topic: D.08. Pain

Title: Effect of joint immobilization by cast on the pain threshold, the itch sensation and the negative component of pain

Authors: *K. NODA, M. OGATA, H. AKITA, H. ISHIBASHI;
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Abstract: Immobilization of joint by cast is commonly used for resting the injured joint, but it often causes the reduction of pain threshold for mechanical stimuli. We have reported that the hyperalgesia induced by intraperitoneal injection of paclitaxel, one of the chemotherapeutic agents clinically used to treat several types of cancer, reduced the negative affective component of pain using a conditioned place aversion test (Noda et al., 2014). However, it remains unclear whether the cast-immobilization affects the emotional component of pain. Furthermore, although itching is a frustrating problem for a person with a cast, little is known about the effect of cast-immobilization on itch-associated response in experimental animals. In this study using rat, therefore, we examined whether the hypersensitivity induced by cast immobilization alters the

pain-induced place aversion and modulates itch sensation. To examine the effects of cast immobilization on pain- and itching-behaviors in rats, one hind limb was wrapped with a wire mesh to keep the ankle joint almost straight for 2 weeks and observation of behavior was conducted after cast removal for 3 weeks. The pain perception threshold to mechanical stimulation was measured by using calibrated von Frey filament test before and after cast immobilization. The joint immobilization elicited the reduction of the paw withdrawal threshold which continued almost over 10 days after cast removal. The serotonin-induced itch-associated response including licking and biting behavior was escalated when compared with sham-operated control rat. Surprisingly, the formalin-induced conditioned place aversion was decreased in the rats with the cast treatment. These results suggest that the joint-immobilization by the cast not only reduce the mechanical pain threshold but also facilitates itch sensation. On the other hand, the cast-immobilization may disturb to form the paired-associate learning between the painful stimulation-induced aversive emotion and the pain-conditioned environment.

Disclosures: **K. Noda:** None. **M. Ogata:** None. **H. Akita:** None. **H. Ishibashi:** None.

Poster

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Topic: D.08. Pain

Support: Catharine Sharpe Foundation

Title: Radiculopathy induces spontaneous and reflex pain that is attenuated by meloxicam in a rat model of nerve root trauma

Authors: C. WEISSHAAR[1], B. PHILIPS[1], *B. A. WINKELSTEIN[2];
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Abstract: Many rodent pain studies use reflex testing with mechanical and thermal stimuli to evoke pain responses. Yet, these testing methods do not measure aspects of spontaneous pain, particularly in the early time period following injury. The rat grimace scale (RGS) quantifies spontaneous pain after inflammatory, visceral, incision, and orthopedic injuries, incorporating observational ratings based on positioning of the eyes, nose, whiskers, and ears. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to relieve inflammatory and post-surgical pain in both human and veterinary patients. However, the effects of NSAIDs on spontaneous pain after radicular injury are unknown. The goal of this study was to evaluate if the NSAID,

meloxicam, alters spontaneous and reflex pain responses in a rat model of cervical radiculopathy. Accordingly, spontaneous pain behavior was quantified using RGS, and mechanical hyperalgesia was measured by reflex testing on the forepaw, after a transient C7 right dorsal nerve root compression (NRC; n=7). A separate group received meloxicam (2mg/kg in 1ml saline) subcutaneously immediately prior to application of the root compression (NRC+meloxicam; n=7). As controls, another group underwent surgery but no compression (sham; n=7). RGS was measured at baseline (before injury), and 3, 6, 24 and 48 hours after injury; ipsilateral forepaw mechanical hyperalgesia was assessed at baseline, and days 1, 3, 5, and 7. The RGS scores of NRC at 6 hours are significantly higher than those for NRC+meloxicam ($p=0.012$) and sham ($p=0.041$). Yet, by 24 hours, the RGS scores for all groups return to baseline, and are not different from each other. Meloxicam also significantly ($p<0.001$) attenuates mechanical hyperalgesia at days 1, 3, and 5 compared to a compression without treatment. Interestingly, despite the fact that RGS scores for all groups returned to baseline by 24 hours, overall mechanical hyperalgesia is significantly reduced by meloxicam treatment ($p<0.001$). Together, these results suggest that pre-treatment with an NSAID reduces both spontaneous and reflex pain behaviors in the early stages of radicular pain and longer-term reflex pain. However, the first 6 hours after injury, and in pain development, may be a critical window in which to capture elements of spontaneous pain by RGS after neuropathic injury. Although additional studies are needed to evaluate long-term spontaneous pain after radicular injury, these findings provide a better understanding of spontaneous pain in a rodent model of radiculopathy and may also offer a foundation for more effective timing in the treatment for radicular pain.

Disclosures: C. Weisshaar: None. **B. Philips:** None. **B.A. Winkelstein:** None.

Poster

064. Pain Models: Behavior II

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Topic: D.08. Pain

Support: SAPPI: 20101087

Title: Involvement of opioid or GABA systems in the ventrolateral periaqueductal gray on analgesia associated to tonic immobility

Authors: *A. M. PÁEZ[1,2], P. VÁZQUEZ LEÓN[2], C. CAMPOS-RODRÍGUEZ[2], E. RAMÍREZ SAN JUAN[2];

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Abstract: Ventrolateral periaqueductal gray (VL-PAG) contains key neuronal circuits related to analgesia and integrated defensive behaviors such as immobility response (IR) which is characterized by a reversible state of motor inhibition. One form of IR can be elicited in rat by touch, labyrinthine stimulation and restriction of movements called tonic immobility (TI) specifically immobility by clamping the neck (ICN). It is known that IR induced-analgesia could be elicited by manipulations or drugs acting at different levels of CNS. The aim of this study was assess the role of either opioid or GABA systems in the TI elicited-analgesia, particularly into VL-PAG. In present work, TI induced-analgesia in naive rats was assessed by tail-flick (TF) test, opioid systemically or into VL-PAG and GABAergic involvement into VL-PAG associated to TI induced-analgesia was assessed. We found that TI elicited-analgesia in naive rats, morphine either systemic or within VL-PAG increased both TF latency and TI duration separately and as well in time duration when occurred both responses simultaneously. On the other hand, naloxone injected systemically or locally into VL-PAG blocked significantly TI elicited-analgesia. Microinjection of muscimol into VL-PAG significantly reduced both TF latency and TI duration when occurred simultaneously, and bicuculline increased TF latency showing an analgesic effect when asses only TF, but not modified TI duration time when both responses occurred simultaneously. These data suggest that TI produce analgesia in rat; it is mediated by opioids at different levels of CNS but critically into VL-PAG, mainly by inhibition of intrinsic tonic GABAergic activity. The sum of effects of morphine or bicuculline and TI is unable to upgrade analgesic response or made it greater than likely, means certain upper limit in such conditions.

Disclosures: A.M. Páez: None. **P. Vázquez León:** None. **C. Campos-Rodríguez:** None. **E. Ramírez San Juan:** None.

Poster

064. Pain Models: Behavior II

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FAPERGS/PRONEM # 11/2050

MCT/FINEP – COENG/2013

Title: Transcranial direct current stimulation reverses hyperalgesia and alters cytokines in neuropathic pain model

Authors: *I. L. TORRES[1,2,3], S. G. CIOATO[1,4], L. F. MEDEIROS[1,2], P. MARQUES FILHO[1], R. VERCELINO[1], A. SOUZA[1], V. L. SCARABELOT[1,5], C. OLIVEIRA[1], L. S. ADACHI[1], J. R. ROZISKY[1], W. CAUMO[1];
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Abstract: Neuropathic pain (NP) is caused by a primary insult or dysfunction in the peripheral or central nervous system. The main symptoms are mechanical allodynia and hyperalgesia to both mechanical and thermal stimuli. NP often shows insufficient response to classic analgesics and remains a challenge to medical treatment and scientific research. The transcranial direct current stimulation (tDCS) is a non-invasive method of cerebral stimulation and represents a promising resource to pain management since it promotes neuroplasticity in the central system of pain and can be combined with other interventions. The aim of this study was to investigate the effects of tDCS in thermal and mechanical hyperalgesia induced by a NP model and in IL-1β, IL-10, and TNF-α levels in central nervous system structures. The chronic constriction injury (CCI) of sciatic nerve was used for the induction of NP. After the establishment of NP, the animals of treated groups were subjected to a 20 minutes session of anodal tDCS, every afternoon for eight days. The thermal hyperalgesia and mechanical allodynia were assessed by Hot plate (HP) and Von Frey (VF) tests, respectively, and evaluated on baseline, 7 and 14 days after surgery; immediately, 24 hours and 7 days after treatment. The IL-1β, IL-10 and TNF-α cortex, spinal cord and brainstem levels were determined by sandwich-ELISA at 48 hours and 7 days after the end of treatment. CCI model causes thermal and mechanical hyperalgesia until at least 30 days after the surgery; however, the anodal tDCS treatment was able to relieve the nociceptive behavior for up to 7 days after the treatment end. In summary, we showed that anodal tDCS is effective to relieve neuropathic pain and modulate cytokine in CCI rat model, and its effect is observed at long-term. Additionally, we observed an important role of the central immune system in the neuropathic process, which can be involved with the maladaptative neuroplastic changes.

Disclosures: **I.L. Torres:** A. Employment/Salary (full or part-time):; Universidade Federal do Rio Grande do Sul. Other; Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq). **S.G. Cioato:** None. **L.F. Medeiros:** None. **P. Marques Filho:** None. **R. Vercelino:** None. **A. Souza:** None. **V.L. Scarabelot:** None. **C. Oliveira:** None. **L.S. Adachi:** None. **J.R. Rozisky:** None. **W. Caumo:** None.

Poster

064. Pain Models: Behavior II

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Topic: D.08. Pain

Support: NIH Grant AR057194

Title: Itch-related scratching and alloknesis elicited by cinnamaldehyde in mice

Authors: *E. E. CARSTENS, M. IODI CARSTENS, T. NGUYEN;
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Abstract: Scratching elicited by many non-histaminergic itch mediators requires coexpression of TRPA1 in pruriceptive nerve endings. The TRPA1 agonist cinnamaldehyde (CA) elicited itch in human skin (Namer et al., Neuroreport 16:955-9, 2005). We investigated if CA elicits itch-related scratching behavior in mice. Using C57BL6 mice, CA (1-40%) suspended in 5% or 10% Tween80 was delivered unilaterally to the cheek via intradermal injection (10 μL) or topical application. Animals were videotaped for 40 min and the number of discrete hindlimb scratch bouts or forelimb wipes directed to the treated cheek, as well as bilateral facial groom bouts, were scored offline by blinded observers. To test for alloknesis, 40% CA was applied to the rostral back (~1 mm2 area). At 5-min intervals, a von Frey monofilamant (0.7 mN bending force) was applied 3 times to mid- and lateral portions of the treated skin, and the occurrences of immediate hindlimb scratch bouts directed to the stimulus were counted to generate an alloknesis score (# scratch bouts/3). We also counted touch-evoked “wet dog shakes”. Intradermal cheek injection of CA elicited a significant ($F=3.2$, $p<0.05$, ANOVA), dose-dependent increase in number of scratch bouts, reaching a plateau at 5%. Scratching peaked at 10-25 min post-CA and then declined. No dose of CA significantly affected the number of forelimb wipes or groom bouts compared to vehicle injections (except reduced grooming at 20% CA). Topical cheek application of CA (30, 40%) did not elicit significant increases in hindlimb scratch bouts or forelimb wipes compared to vehicle treatments (all counts <10 per 40 min). However, mice exhibited significantly fewer groom bouts following 30 and 40% CA compared to vehicle.

Topical application of 40% CA to the rostral back did not elicit scratching per se. There was a trend toward increased alloknesis score ($p=0.077$, paired t-test) and a significant increase in wet dog shakes ($p<0.005$, paired t-test) vs. vehicle application. Touch-evoked wet dog shakes may represent another sign of alloknesis. Vehicle treatment also increased alloknesis scores starting 10 min post-application, but to a lesser extent than CA. CA presumably acts at TRPA1 in cutaneous nerve endings to elicit scratching and alloknesis. Scratching peaked at 5% CA, strikingly similar to the report that 5% CA elicits maximal itch on human skin (Holand et al., Acta Derm Venerol 2015). Another TRPA1 agonist, mustard oil, elicited wiping but not scratching (Spradley et al., Acta Derm Venereol 92:515-20, 2012). This suggests that there is a narrow concentration range over which TRPA1 agonists selectively activate pruriceptors.

Disclosures: E.E. Carstens: None. **M. Iodi Carstens:** None. **T. Nguyen:** None.

Poster

064. Pain Models: Behavior II

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Topic: D.08. Pain

Title: Is the chronic constriction injury model in the mouse an adequate screening model for analgesic activity?

Authors: *D. PUSHETT, E. ESNEAULT, C. LE CUDENNEC, B. HELARD, V. CASTAGNE; Porsolt SAS, Le-genest-saint-Isle, France

Abstract: Different models of neuropathies have been established in the rat and their effects after nociceptive stimulations are extensively described in the literature. However, similar murine models may be useful for proof-of-concept studies with transgenic animals or if there is limited availability of test substance. The objective of this study was to establish a neuropathic model in mice and to validate it pharmacologically. Mice were operated according to a modified protocol of the standard chronic constriction injury (CCI)/Bennett (i.e. three tied ligatures in the mouse instead of four in the rat). The mice (n=10) were then subjected to the electronic von Frey test to confirm the presence of tactile allodynia. The mice were subsequently evaluated using 3 additional pain endpoints: thermal and tactile hyperalgesia and thermal allodynia, using the plantar, pinchmeter, and cold plate tests, respectively. Analgesic effects of morphine and tramadol were investigated in this model. In the electronic von Frey test, the force inducing paw withdrawal was significantly decreased in the lesioned paw as compared to the non-lesioned paw and this difference was observed up to 25 days post-surgery. Morphine displayed an analgesic

effect whereas tramadol was inactive. In the plantar test, we observed significant differences between both hindpaws and significant effects of morphine and tramadol, however, we do not consider this test to be a robust enough evaluation in this model. In the pinchmeter test (alternative to the Randall & Selitto test) the force inducing withdrawal was significantly different between both paws. However this difference was not observed to persist for as long as tactile allodynia. Morphine and tramadol displayed analgesic effects against tactile hyperalgesia. In the cold plate test, sciatic nerve ligature resulted in the appearance of paw-withdrawal responses when a cold stimulus was applied to the lesioned paw of vehicle controls, whereas no responses were observed in the non-lesioned paw, indicating cold allodynia. However, neither morphine nor tramadol displayed significant analgesic effects in our experimental conditions. These results suggest that CCI surgery in the mouse clearly induces neuropathic pain and that electronic von Frey and pinchmeter tests can be used as endpoints in this model. The CCI model in the mouse is therefore a good model for screening for analgesic activity measured by tactile allodynia or hyperalgesia, prior to follow-up testing in the rat with additional endpoints.

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Poster

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Title: Pain transmission within peripheral sensory ganglia

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Bacteriology, Okayama Univ. Grad. Sch. of Medicine, Dent. and Pharmaceut. Sci., Okayama, Japan; [4]Dept. of Orthodontics, Univ. of Florida, Gainesville, FL

Abstract: Many neuropathic pain patients suffer severe pain; however, available therapies are largely ineffective or inadequate. Peripheral nerve injury induces neuropathic pain states and hyperexcitability of neurons within sensory ganglia. Despite the absence of synaptic contacts in adult sensory ganglia, somata of sensory neurons can be transiently depolarized and cross-excited by activation of neighboring neurons within the same ganglion. In our previous *in vitro* study, neurotransmitter release from peripheral sensory ganglia was inhibited by purified botulinum neurotoxin type A (BoNT/A) administration. In the present study, we sought to modulate neurotransmitter release by direct application of BoNT/A to sensory ganglia in an animal model of neuropathic pain. We evaluated the pain behavior and motor behavior after the BoNT/A application. The peripheral neuropathy site was the right sciatic nerve (SNE). A flexible polyethylene cannula was attached to the L3 transverse process with cyanoacrylate adhesive, and the tip of the cannula was so placed to slightly touch the L4 dorsal root ganglia (DRG). BoNT/A was applied to the L4 DRG via this cannula. We measured the thermal stimulus threshold value of pain and the mechanical stimulation threshold to evaluate pain behavior. Thermal sensitivity testing was performed using the Hargreaves apparatus which measures the withdrawal latency from a radiant heat source directed at the proximal half of the plantar surface of each hindpaw. Mechanical sensitivity was assessed using the electronic von Frey hair pressure transducer. Also, we evaluated general motor function effects using a rotor rod apparatus following BoNT/A application. We measured the pain behavior and motor function of the rats before and after neuropathy surgery and BoNT/A administration. The main findings of this study are as follows: (i) SNE decreased the hindpaw withdrawal threshold to mechanical and withdrawal latency to thermal stimulation, and the direct application of BoNT/A to the L4 DRG counteracted these effects; (ii) The direct application of BoNT/A to the L4 DRG did not affect motor function.

Disclosures: **K. Omoto:** None. **K. Maruhama:** None. **Y. Yamamoto:** None. **T. Sugimoto:** None. **O. Matsushita:** None. **J.K. Neubert:** None. **Y. Matsuka:** None.

Poster

064. Pain Models: Behavior II

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 64.18/M32

Topic: D.08. Pain

Support: NMRC/1255/2010

NMRC/CBRG/0050/2013

Title: A role for neurokinin neurotransmission in forebrain septal region in nociception

Authors: *S. NG, S. KHANNA;
Physiol., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: The septal region is implicated in affect. In context of nociception, both the medial (MS) and lateral septum (LS) have direct and indirect anatomical connections to cortical and sub-cortical regions, including the hippocampus and amygdala, which are key structures involved in the affective component of pain. Previously, we showed that the MS region mediates changes in the hippocampal pyramidal cell excitability and the nociceptive behaviors evoked on hind paw injection of the inflammatory algogen, formalin. In contrast, the role of LS in nociception is not well studied. Neurokinin-1 receptors (NK1R) are robustly expressed in the soma and dendrites of neurons in both the MS and LS. It is well established that spinal NK1R expressing neurons are critical in facilitating nociception. However, the role of septal neurokinins (NK) in acute and chronic pain remains unexplored. In the present study, we have investigated whether NK neurotransmission in the septal region influences indices of pain in male Sprague-Dawley rats. The role of NKs was inferred by investigating the effects of intraseptal microinjection of the NK1R antagonist, L-733,060 (0.0176 µg/µl and/or 0.176 µg/µl; 0.5 µl), on indices of nociception in the formalin model and the chronic constriction injury (CCI) model of neuropathic pain. In anaesthetized rats, microinjection of L-733,060 in MS, but not LS prevented or reversed formalin-induced suppression of hippocampal pyramidal cell excitability. However, formalin-induced theta activation was not altered by intraseptal L-733,060 in both anaesthetized and awake rats. On the other hand, L-733,060 microinjection in both MS and LS attenuated flinching, but not licking of the injured paw in phase two of the formalin test. In the context of CCI, intraseptal L-733,060 attenuated both mechanical allodynia and thermal hyperalgesia. In addition, L-733,060 administered into both MS and LS suppressed pERK activation in central amygdala that was normally observed in parallel with thermal hyperalgesia. These findings suggest that NK neurotransmission in both MS and LS mediates nociception. Especially, NK neurotransmission in the region sustains neuropathic peripheral hypersensitivity and the reflexive withdrawal of injured paw in the formalin model.

Disclosures: S. Ng: None. **S. Khanna:** None.

Poster

064. Pain Models: Behavior II

Location: Hall A

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Topic: D.08. Pain

Support: NMRC/1255/2010

NMRC/CBRG/0050/2013

Title: A role for septal glutamatergic network in modulation of nociception

Authors: *K. IBRAHIM, S. KHANNA;
Physiol., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: The forebrain region medial septum (MS) has been implicated in nociception. Indeed, we have reported previously that the inactivation of neural transmission in the MS led to an attenuation of nociception in the formalin model of persistent inflammatory pain. The MS region has a rich milieu of neurons that includes both intrinsic and projection glutamatergic neurons. The region, in addition, receives glutamatergic afferents. Evidences indicate that glutamatergic terminals in MS synapse with almost all type of neurons in the MS. Thus, the glutamatergic transmission in MS is likely to be ubiquitous. However, the role of the septal glutamatergic transmission in pain still remains unexplored. In the present study, we have investigated whether glutamatergic neurotransmission in the septal region influences nociception in male Sprague-Dawley rats. The role of glutamate was inferred by investigating the effects of intraseptal microinjection of NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione; 10µg/µl-40µg/µl in 0.5µl), an antagonist at the AMPA glutamate receptor, on indices of nociception in the formalin model and the chronic constriction injury (CCI) model of neuropathic pain. Intraseptal microinjection of NBQX attenuated the hippocampal theta activation in a dose-dependent fashion in both anaesthetized and awake rat. A peak effect of NBQX on formalin (1.25%)-induced power of theta wave activity was observed at dose of 20µg/µl. Characterization in anaesthetized rat indicated that the dose of 20µg/µl was selective insofar it did not affect suppression of CA1 population spike even though it attenuated the power of theta evoked on indirect and direct stimulation of the MS. However, NBQX (20µg/µl) attenuated only flinching, but not licking of the injured paw in both phase 1 and 2 of the formalin test. In addition, the agent also partially attenuated peripheral hypersensitivity in the CCI model. Microinjection of NBQX at a higher dose (40µg/µl), though, affected both flinching and licking in the formalin test and reverse peripheral hypersensitivity in CCI model. These results suggest that glutamatergic neurotransmission in MS is recruited in nociception and sustains the nociceptive drive.

Disclosures: K. Ibrahim: None. **S. Khanna:** None.

Poster

064. Pain Models: Behavior II

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 64.20/M34

Topic: D.08. Pain

Support: KAKENHI 26893243

Title: A new pre-clinical animal model for oral ulcer pain following orthodontic wire appliance

Authors: M. ITO, K. ONO, S. HITOMI, K. YAMAGUCHI, T. KAWAMOTO, *K. INENAGA;
Kyushu Dent. Univ., Kitakyushu, Japan

Abstract: Orthodontic appliances frequently cause oral mucosal lesions, resulting in bacterial colonization, gingival inflammation and pain. However, little study has reported about the mechanisms underlying oral ulceration and subsequent pain induction in the orthodontic appliances. In the present study, we developed a new pre-clinical rat model for oral ulcer pain following an orthodontic wire appliance and evaluated ulceration in the oral mucosa and pain-related behavior. Under pentobarbital anesthesia, 8 week-old Wistar rats were installed with an arched orthodontic thick wire of 10 mm length between inferior incisors, soldered to a ligature wire. The sharp tip of the thick wire directly touched the labial fornix region of the oral mucosa. As control, a shorter thick wire (4 mm) was installed similarly without any contacts of the tip on the oral mucosa. Next day (day 1), severe oral ulcer was observed in the model rats and the wire was removed. The wire-induced oral ulcer was cured completely until day 5 after the procedure. In control group, there were no mucosal lesions. To evaluate pain induction in the model, we observed spontaneous mouth rubbing and facial wiping behaviors for 10 min on pre-procedure day and days 1-3 after the procedure. Compared with control group, the both behaviors were significantly enhanced in the oral ulcer model on day 1. The new pre-clinical animal model for orthodontic wire-induced oral ulcer pain can be utilized in evaluations of new drug treatments for oral ulcer pain in dental practices.

Disclosures: M. Ito: None. **K. Ono:** None. **S. Hitomi:** None. **K. Yamaguchi:** None. **T. Kawamoto:** None. **K. Inenaga:** None.

Poster

064. Pain Models: Behavior II

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Program#/Poster#: 64.21/M35

Topic: D.08. Pain

Support: SFI 10/IN.1/B2976

Title: Genotype-dependent exacerbation of nerve injury-induced pain, anxiety and depressive behaviour in rats

Authors: E. M. JENNINGS[1,2,3], N. N. BURKE[1,2,3], M. ROCHE[4,3,2], *D. P. FINN[3,1,2]; [1]Pharmacol. and Therapeut., [2]Ctr. of Pain Res., [4]Physiol., [3]Natl. Univ. Ireland, Galway, Galway, Ireland

Abstract: Numerous studies indicate a very high prevalence of chronic pain comorbid with anxiety disorders and depression (Asmundson and Katz, 2009, Bair et al., 2003). The relationship between altered emotional states and chronic pain disorders is complex and the bidirectional interplay between affective disorders and chronic pain is poorly understood. Wistar-Kyoto (WKY) rats are an inbred rat strain that demonstrate hyper-responsivity to stress, a depressive and anxiogenic phenotype, as well as enhanced nociceptive responding to visceral (O'Mahony et al., 2013), mechanical and inflammatory (Burke et al., 2010, Rea et al., 2014) stimuli, compared with Sprague-Dawley (SD) rats. The aim of this study was to investigate if WKY rats, as a model of trait negative affect, exhibit altered nociceptive behaviour and depressive- or anxiety-like behaviour following peripheral nerve injury, compared with SD counterparts. L5 spinal nerve ligation (SNL) resulted in prolonged (up to 30 days) mechanical and cold allodynia in adult male SD and WKY rats as measured by the von Frey and acetone drop tests, respectively. Prolonged SNL-induced heat hyperalgesia in the Hargreaves test was only observed in WKY, but not SD rats. Baseline testing showed that WKY rats displayed increased anxiety-like behaviour (reduced time in centre zone of the open field) compared to the SD rats. Post-SNL, both SD and WKY rats displayed increased anxiety-like behaviour compared to sham counterparts, but levels of anxiety-like behaviour were higher in WKY rats than in SD rats. WKY-sham rats spent more time immobile in the forced swim test versus SD-sham rats, indicating increased depressive-like behaviour. Immobility was greater in the WKY-SNL group versus the WKY-sham group, while no difference in immobility was observed between SD-sham and SD-SNL groups. In conclusion, these data suggest increased sensitivity to noxious heat, and increased anxiety- and depressive-like behaviour following peripheral nerve injury in a genotype (WKY) predisposed to negative affect.

Disclosures: E.M. Jennings: None. **N.N. Burke:** None. **M. Roche:** None. **D.P. Finn:** None.

Poster

064. Pain Models: Behavior II

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Topic: D.08. Pain

Support: TWU Research Enhancement Program Grant

Title: Peripheral serotonin evokes sexually dimorphic and estrous cycle dependent pain behaviors in male and female rats

Authors: W. L. BENTON[1], Y. J. LEE[1], R. H. BESHER[1], *D. L. AVERITT[2];
[1]Biol., [2]Texas Woman's Univ., Denton, TX

Abstract: Many persistent pain conditions occur predominantly in women making pain a major women's health issue. As the majority of pain research has been conducted in males, the mechanisms underlying pain in women are unclear. One theory for the prevalence of pain disorders in females is steroid hormone modulation of pain mechanisms. The monoamine neurotransmitter serotonin (5HT) has been highly implicated in various pain conditions that are more prevalent in women. Our recent studies found that peripheral 5HT (1) evokes pain via a subpopulation of trigeminal nociceptors expressing the transient receptor potential V1 channel (TRPV1) and (2) potentiates capsaicin-evoked proinflammatory peptide release from human tissue during the luteal phase of the menstrual cycle. No studies have yet examined the effect of steroid hormone modulation on 5HT-evoked pain behaviors in female rats. We hypothesized that peripheral 5HT evokes greater pain sensitivity in female rodents during stages of the estrous cycle when steroid hormones are fluctuating. Female Sprague-Dawley rats (250-350 g) from each stage of the estrous cycle (proestrus, estrus, diestrus 1 or diestrus 2) and intact males were acclimated to the testing apparatuses. Vaginal lavages were performed daily to determine the stage of estrous at the time of testing. Rats received an intraplantar hindpaw injection of 5HT (2 μg / 100uL) or saline (n = 6 per stage of estrous and sex) and pain behaviors were measured at 0, 10, 20 and 30 minutes post-injection. Thermal hyperalgesia was detected by measuring changes in paw withdrawal latencies to a noxious thermal stimulus using the Plantar Test (Ugo Basile). Mechanical allodynia was detected by measuring changes in force in grams to elicit paw withdrawal using the Dynamic Plantar Aesthesiometer (Ugo Basile). Here we report that female rats in proestrus and estrus exhibited significantly greater thermal hyperalgesia within 10 minutes of 5HT injection compared to males and diestrus females. Proestrus and estrus females also retained significant hyperalgesia at 20 minutes, while males and diestrus females returned to baseline. These data provide evidence of a modulatory role of steroid hormones on 5HT-evoked pain, which may underlie the greater prevalence of pain disorders in women. Our ongoing

studies are aimed at delineating the role of various 5HT receptors and the possible interaction with TRPV1 in female rats.

Disclosures: **W.L. Benton:** None. **Y.J. Lee:** None. **R.H. Besher:** None. **D.L. Averitt:** None.

Poster

065. Somatosensory Thalamocortical Processes

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Topic: D.09. Tactile/Somatosensory Systems

Support: NIH Grant R01NS085447

NIH Grant R01NS48285

NIH Grant U01MH106027

Title: Controlling transmission of sensory information by optical manipulation of the thalamo-cortical pathway in the anesthetized mouse

Authors: ***P. BORDEN**, A. D. ORTIZ, H. J. V. ZHENG, G. B. STANLEY;
Georgia Tech., Atlanta, GA

Abstract: The thalamus plays a vital role in processing and integrating both sensory afferent and motor efferent signals. Although previous work has shown that thalamic state has a dramatic effect on ongoing cortical state, it is not yet clear how this alters the transmission of sensory information to the cortex. The thalamic ventral posterior medial (VPm) region is a whisker driven excitatory region, which receives modulatory, polarizing inputs from many regions including cortex, brainstem, and other thalamic nuclei such as reticular thalamus, all of which may interact to set thalamic state and set the degree of synchronization and burst/tonic firing. Here, we directly determined how thalamic membrane potential alters downstream cortical processing of sensory signals, using a combination of electrophysiological recordings and optogenetic techniques in the anesthetized mouse lemniscal pathway. We performed simultaneous extracellular recording of thalamic (VPm) single units and wide-field voltage sensitive imaging of cortical spatiotemporal activity (S1 Barrel Field) while modulating thalamic membrane potential and stimulating the single whisker of the anesthetized mouse. We virally transfected young mice with optogenetic modulatory channels (ChR2, and eNph3.0) for direct manipulation of thalamic membrane potential. Our preliminary results suggest that the ongoing thalamic membrane potential dramatically alters the corresponding evoked cortical

spatialtemporal activity in a nonlinear fashion. In particular, by hyperpolarizing the thalamic membrane immediately prior to whisker stimulation, there is an observed increase in the corresponding cortical spatial response for all sensory stimuli. Additionally, hyperpolarization of the VPm thalamic membrane appears to shift the cortical velocity response curve by increasing the sensitivity to lower velocity stimuli. This effect, therefore is not simply adjusting the gain of the sensory information, but altering how sensory stimuli are encoded and transmitted to the cortex. This work is further evidence that the thalamus may not act as a passive relay, but instead as a dynamic filter, that controls how information is encoded and processed.

Disclosures: **P. Borden:** None. **A.D. Ortiz:** None. **H.J.V. Zheng:** None. **G.B. Stanley:** None.

Poster

065. Somatosensory Thalamocortical Processes

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Title: Information coding through adaptive control of synchronized thalamic bursting

Authors: *C. WHITMIRE[1], C. WAIBLINGER[1], C. SCHWARZ[2], G. STANLEY[1];
[1]Wallace H Coulter Dept. of Biomedial Engin., Georgia Tech/Emory, Atlanta, GA; [2]Hertie Inst. for Clin. Brain Res., Univ. of Tübingen, Tübingen, Germany

Abstract: Beyond performing as a simple relay from the periphery to cortex, the thalamus acts as a “gate” for the peripheral signals, controlling what does and does not get transmitted to cortex. Furthermore, this gating is dynamic, and can be influenced through both bottom-up sensory influence and top-down mechanisms related to wakefulness and attention. In this work, we explored the bottom-up effect of stimulus adaptation on the encoding of sensory features in the whisker thalamocortical circuit of the fentanyl-cocktail anesthetized rat. Previous work has

demonstrated that adaptation can lead to enhanced discriminability paired with reduced detectability, but the underlying mechanism is only beginning to be studied. In the context of a sensory adaptation paradigm, we investigated the role of the level of adaptation, as quantified by the strength of the adapting input, on thalamic spiking, burst spiking, and synchronous firing. Increasing levels of adaptation reduced the amplitude of the evoked response and effectively shifted thalamic neurons from burst to tonic firing when conveying information related to an embedded sensory signal. Furthermore, increasing adaptation led to reduced levels of synchrony across pairs of simultaneously recorded neurons. These experimental results demonstrate that thalamic cells fire more burst spikes in response to "signals" presented in isolation than in noise and that this leads to a higher detectability, but a lower discriminability, as assessed using an ideal observer analysis of the thalamic unit spiking activity. Direct depolarization of the thalamic neurons using channelrhodopsin was also able to shift the encoding of sensory features from burst to tonic spikes. We developed an integrate and fire model neuron with an incorporated burst mechanism to investigate the role of depolarization on thalamic encoding. Consistent with the experimental findings, the model suggests that the sensory adaptation is depolarizing the membrane potential of the simulated cell and that this is sufficient to explain the shift in bursting. Taken together, these results suggest that the level of sensory adaptation may have a sustained depolarization effect that dynamically gates information flow through modulations to the sensory evoked response, the burst spiking activity, and the synchrony across neurons. Furthermore, these results could have broad implications for a more comprehensive coding strategy whereby ongoing sensory stimulation, as experienced constantly in natural sensing conditions, dynamically alters the state of the thalamus to fundamentally shape the functional encoding of the pathway.

Disclosures: **C. Whitmire:** None. **C. Waiblinger:** None. **C. Schwarz:** None. **G. Stanley:** None.

Poster

065. Somatosensory Thalamocortical Processes

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Topic: D.09. Tactile/Somatosensory Systems

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NSF CRCNS Grant IOS-1131948

NIH 2R01-NS-048285

Title: Thalamic control of sensory perception in the awake behaving rat

Authors: *C. WAIBLINGER[1,2,3], C. J. WHITMIRE[1], C. SCHWARZ[3,2], G. B. STANLEY[1]; [1]Wallace H Coulter Dept. of Biomed. Engin., Georgia Tech. and Emory, Atlanta, GA; [2]Dept. of Cognitive Neurol., Hertie Inst. for Clin. Brain Res., University of Tübingen, Germany; [3]Systems Neurophysiol., Werner Reichardt Ctr. for Integrative Neurosci., University of Tübingen, Germany

Abstract: The rodent ventro-posterior-medial nucleus (VPm) is the thalamic station of ascending whisker signals and has been viewed as the gateway for sensory information to enter the barrel cortex. Whether simple sensory detection or discrimination tasks can be processed subcortically is still hotly debated. Here, we set out to investigate how different sensory inputs are encoded by VPm neurons and whether thalamic processing can modulate sensory perception. Our previous work on the whisker thalamocortical circuit of the anesthetized rat suggests that sensory adaptation dynamically gates information flow through modulation of the sensory evoked response, elicited burst spiking activity and the synchrony across neurons. Using a similar signal-in-noise paradigm with precise whisker stimuli that mimic whisker vibrations upon texture contact, we trained head-fixed rats to discriminate fast kinematic features (tagged 'slip-like events') from background-noise at the level of their perceptual threshold (equal number of hit and miss trials) while simultaneously recording in the principal VPm barreloid. We found that isolated single units encoded slip-like events by fast transient responses, showing elicited bursts in a fraction of trials. Introducing background-noise reduced the sensory evoked response and burst activity similar to the findings in the anesthetized preparation. When investigating multi-unit population activity regarding the animal's behavioral response, we found that spiking activity differed with respect to hit versus miss trials in prolonged time periods. Spike counts were lower before and higher after presentation of the reward associated stimulus in hit compared to miss trials, suggesting an ongoing thalamic influence on the animal's response probability. Taken together, our results confirm the finding in the anesthetized animal that thalamic processing is significantly altered by adapting sensory signals and further elaborates the idea that sensory perception is partially determined by subcortical structures.

Disclosures: C. Waiblinger: None. **C.J. Whitmire:** None. **C. Schwarz:** None. **G.B. Stanley:** None.

Poster

065. Somatosensory Thalamocortical Processes

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Topic: D.09. Tactile/Somatosensory Systems

Support: CIHR

GRSNC

FRQS

Title: Effects of spatial and cross-modal focusing of attention on somatosensory neuronal activity in primate thalamus

Authors: *E.-M. MEFTAH[1], A. CYBULSKA-KLOSOWICZ[2], E. C. CHAPMAN[1];
[1]Neurosciences, Univ. De Montreal, Montreal, QC, Canada; [2]Lab. of Neuroplasticity, Nencki Inst., Warsaw, Poland

Abstract: Focusing our attention enhances perception but knowledge of the underlying neuronal substrates is incomplete. We have shown that selective attention to tactile texture produces a generalized additive increase of texture-related activity in both primary (S1) and secondary (S2) somatosensory cortex and a focused multiplicative increase of texture-related activity in S2 (Meftah et al. 2001; Chapman and Meftah 2005). Using the same paradigm, we extended our recordings to an earlier level of processing, corresponding to the somatosensory thalamus (VPL, ventral posterior lateral nucleus) and showed that attention modulated neuronal responsiveness to textured stimuli, potentially contributing to the modulation seen in S1 and S2 (Cybulska-Klosowicz et al. 2010, SFN). We have now extended our previous investigation to include thalamic cells with a cutaneous receptive field (RF) that was either directly stimulated by the moving textured surfaces (on-focus, tips of digits 3/4) or not (off-focus, elsewhere). Our working hypothesis was that attention would enhance inputs critical for task performance and also suppress irrelevant cutaneous inputs (off-focus). Single unit recordings were made from VPL while a trained macaque performed a cross-modal attention task, attending and discriminating changes in tactile roughness or light intensity. Competing stimuli (tactile and visual) were presented in each trial. Attention was manipulated by an instruction cue prior to each trial. Recordings were made from 38 cells with a cutaneous RF, 21 on-focus and 17 off-focus RFs. The former showed an increase in discharge as textured surfaces were displaced under the fingertips, and were frequently texture-related (12/21). Our results confirmed that attentional modulation is frequently encountered for on-focus VPL cells (11/21), with discharge being enhanced with directed attention (10/11). The discharge of 14 off-focus cutaneous neurones was modulated during tactile stimulation, but most showed decreased discharge (8/14); none were texture-related. A small proportion of off-focus neurones were modulated by directed attention (5/17) but the sign of the effect was mixed (3 decrease, 2 increase). For both on- and off-focus cells, the attentional effects were concentrated in the texture change period. These results confirm that selective attention modulates neuronal responses to tactile stimuli at a very early stage of processing, VPL. In addition, a filtering process suppresses cutaneous information not

relevant for the task providing a spatial focus on the digit tips, the source of information critical for detecting the salient change in roughness.

Disclosures: **E. Meftah:** None. **A. Cybulska-Klosowicz:** None. **E.C. Chapman:** None.

Poster

065. Somatosensory Thalamocortical Processes

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Support: NSERC

CIHR

GRSNC

FRQS

Title: Vibrotactile perception is enhanced by anodal transcranial direct current stimulation, tDCS, of primary somatosensory cortex, S1

Authors: ***S. LABBÉ**, E.-M. MEFTAH, E. C. CHAPMAN;
Univ. De Montréal, Montreal, QC, Canada

Abstract: tDCS is a non-invasive technique whereby weak, direct current stimulation is applied to the cortex to enhance, anodal (a), or decrease, cathodal (c), cortical excitability. We previously showed that vibration detection thresholds are decreased during a-tDCS but not c-tDCS (Labbé et al. SFN 2014). Here, we examined the effects of tDCS (a-, c- and sham), applied to the right S1, on vibrotactile discrimination of the amplitude of pairs of 20 Hz vibrations applied to the distal pad of the left middle finger. Performance was measured in 5 blocks of trials: before (2), during (1) and after (2) tDCS (intensity, 1 mA; duration 20 min). All subjects (n=12) participated in 3 sessions (a-, c-, s-tDCS; order counterbalanced). Discrimination threshold was measured in a two-alternative forced choice paradigm. Pairs of stimuli were presented (duration, 0.8 s; interstimulus delay, 0.8 s). The amplitude of the standard stimulus was 50 μm; comparison stimuli of 50, 53, 56 and 59 μm were presented (order counterbalanced). Subjects identified the larger stimulus in each pair (first or second). The mean discrimination threshold at baseline (75% correct) was 7.5 μm (range, 3 - 13.5 μm). There was a significant difference in threshold across sessions during tDCS ($p = 0.027$). Post hoc tests showed that threshold was significantly lower

during both a-tDCS, 15%, and c-tDCS, 14%, vs sham. Overall, 9/12 subjects (responders) showed a decrease during and immediately after a-tDCS; their threshold returned to baseline 30 min later. Threshold varied significantly as a function of group (responder vs non responder) across the 5 blocks of the a-tDCS session, with group explaining 52% of the variance in threshold. Group was not a significant factor for either the c- or the s-tDCS sessions (effect sizes, 2 and 3%). Finally, the non responders had the lowest baseline thresholds of all subjects in the anodal session, 3.8 to 5.5 μm. The lack of effect with a-tDCS may represent a ceiling effect. The present results are mostly in accordance with our previous results for vibrotactile detection thresholds. Together, the results suggest that S1 a-tDCS enhances cortical excitability by shifting the underlying stimulus-response function to the left (decreased detection threshold) and increasing its slope (decreased discrimination threshold).

Disclosures: **S. Labbé:** None. **E. Meftah:** None. **E.C. Chapman:** None.

Poster

065. Somatosensory Thalamocortical Processes

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Minverva

DFG-SFB 1089

President of Israel Prize of Excellence in Neuroscience

Title: Local and thalamic origins of ongoing and sensory evoked cortical synaptic correlations

Authors: ***B. MOHAR**[1,2], K. COHEN-KASHI MALINA[1], I. LAMPL[1];
[1]Weizmann Inst. of Sci., Rehovot, Israel; [2]Janelia Res. Campus, Ashburn, VA

Abstract: The contribution of local circuits versus remote inputs in the generation of synchronized activity in sensory cortices is poorly understood. In sensory cortices, ascending information from the thalamus targets cells in L4. However, the impact of these inputs is controversial. The majority of the synaptic contacts in L4 are between neighboring cortical cells. However, electrophysiological studies have suggested that thalamic inputs are powerful enough to evoke the observed subthreshold response of L4 cells, without requiring inputs from

neighboring cortical cells. How then such a low number of thalamic inputs can have such a profound impact on cortical response? A widely accepted mechanism that explains the robust activation of L4 cells is thalamic synchrony, implying that thalamic inputs of neighboring L4 cells are highly correlated. To address this question we used optogenetic silencing of cortical firing, by expressing ChR2 in inhibitory interneurons. This was done in order to isolate the thalamic inputs of simultaneously whole cell recorded nearby L4 neurons (<200 μm) in the mice barrel cortex. Surprisingly, we found that although on average 46% of the total sensory evoked excitatory current in these cells originates from thalamic cells, thalamic synaptic inputs to L4 cells are not correlated, neither in time nor in magnitude. During ongoing activity the correlation between pairs of cells dropped by an average of 72% upon silencing of cortical firing. Importantly, we found no relation between the trial to trial correlations of sensory evoked excitatory responses (during intact cortical activity) and those of the isolated thalamic inputs. Hence, synchronized activities in nearby L4 cells during spontaneous and sensory evoked activities do not emerge from thalamic inputs. Additionally, we found that the relative thalamic contribution, measured from the average response, was invariant to stimulation conditions, but could be substantially different in the simultaneously recorded cells. In summary, we suggest that nearby L4 cells receive their inputs from independent pools of thalamic neurons which are amplified in a distinct manner in each cell and thus, synchronized activity emerges due to distinct intracortical network properties.

Disclosures: **B. Mohar:** None. **K. Cohen-Kashi Malina:** None. **I. Lampl:** None.

Poster

065. Somatosensory Thalamocortical Processes

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Title: Spatiotemporal dynamics of sensory processing in the rat whisker tactile system

Authors: *D. HIRAI[1], K.-I. SHIBATA[1], K. C. NAKAMURA[1], T. TANAKA[2], H. HIOKI[1], T. KANEKO[1], T. FURUTA[1];
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Abstract: The vibrissa sensory system is a popular model of sensory processing. In the rodent trigeminal pathway, sensory inputs from the face are topographically mapped onto S1, and the orderly arrangement of whiskers on the snout is represented centrally by arrays of cellular aggregates referred to as barrels. By contrast, it is still now unclear how the central nervous system processes time in the early sensory processing. Temporal processing is required for simple sensory problems, such as interval, duration and motion discrimination. In this study, we examined how peripheral sensory input was encoded at cortical and subcortical levels of the vibrissa sensory system. Here, the juxtacellular labeling technique, which allows selective labeling and visualization of individual neurons in which electrophysiological data have been obtained, was applied to recordings. The vibrissa of rats was pushed mechanically by a piezo-driven vibrating insertion device, and neuronal responses to the whisker deflection were analyzed in waking head-restrained rats. As a result, this study revealed several new findings. (1) Subgroups of neurons in thalamic VPM nucleus showed onset and late (50ms-) responses to whisker deflection, and LTS bursts were hypothesized to be the origin of the late response. (2) The reticular nucleus (TRN) provides the only source of GABAergic projections to VPM, and whisker-responsive TRN neurons showed burst discharge in onset period, and the burst firing could induces to suppression prior to late response in VPM. (3) In contrast, less cortical neurons showed late response, and some corticothalamic projection neurons in the barrel column showed short latency sensory response as well as layer 4 neurons as dominant thalamic recipient. (4) By microinjection of GABA, we found effects of S1 inactivation to both onset and late responses of VPM and TRN neurons. In summary, the present experiments reveal that thalamic neurons have not simple relay function, and thalamic sustained spatiotemporal processing could be modulated by corticothalamic feedback during waking state. These observations suggest the thalamus gates peripheral sensory input based on the current cortical demand such as active sensation.

Disclosures: D. Hirai: None. **K. Shibata:** None. **K.C. Nakamura:** None. **T. Tanaka:** None. **H. Hioki:** None. **T. Kaneko:** None. **T. Furuta:** None.

Poster

065. Somatosensory Thalamocortical Processes

Location: Hall A

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Topic: D.09. Tactile/Somatosensory Systems

Support: NIH grant HD060117

NIH R21 NS071225

NIH R01 EY 015387

NIH R21 060117

Title: Early experience is associated with individual differences in brain organization and thalamic connectivity in prairie voles (Microtus ochrogaster)

Authors: *A. M. SEELKE[1], A. PERKEYBILE[2], K. BALES[3], L. KRUBITZER[3];
[1]Dept. of Psychology, Univ. California, Davis, Davis, CA; [2]Psychology, Northeastern Univ., Boston, MA; [3]Psychology, Univ. of California, Davis, Davis, CA

Abstract: Early life sensory experiences have a profound effect on brain organization, connectivity and subsequent behavior. In most mammals, the earliest sensory inputs are delivered to the developing brain through tactile contact with the parents, especially the mother. Prairie voles (Microtus ochrogaster) are monogamous and biparental rodents. Similar to other rodents, the type and amount of parental behavior exists on a spectrum, ranging from low contact (LC) to high contact (HC). However, very little is known about how these early and pervasive differences in tactile stimulation and social experience influence cortical organization and connectivity. To address this question we used neuroanatomical tracing techniques to examine the cortical, callosal, and thalamocortical connections of the primary somatosensory area (S1) in the offspring of HC and LC voles. Injection sites within S1 were matched so that direct comparisons between these two groups could be made. We observed several important differences between these groups. The first was that HC offspring had a greater density of intrinsic connections within S1 compared to LC offspring. The HC offspring had a more restricted pattern of ipsilateral connections while LC offspring had dense connections with areas of parietal and frontal cortex that were more widespread. Additionally, LC offspring had a broader distribution of callosal connections than HC offspring and a significantly higher percentage of callosal labeled neurons. Finally, similar to the connection patterns seen in the cortex, HC voles had a more restricted pattern of connections with a small number of thalamic nuclei. The majority of labeled cells were found in the ventral posterior nucleus (VPM and VPL), and the remainder of labeled cells were found in the posterior nucleus (PO), the ventral lateral nucleus (VL), and the ventral medial nucleus (VM). In contrast,LC voles had a broader distribution of connections with a greater number of nuclei. Labeled cells were found in VP(M+L), PO, VL, and VM, as well as the central lateral nucleus (CL) and paracentral nucleus (PC). To date, this is the first study that examines individual differences in neuroanatomical

connections and suggests that they may be related to natural differences in parental rearing styles associated with tactile contact. Ultimately, the population variation generated by these individual differences may play a role in the process of natural selection.

Disclosures: **A.M. Seelke:** None. **A. Perkeybile:** None. **K. Bales:** None. **L. Krubitzer:** None.

Poster

065. Somatosensory Thalamocortical Processes

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 65.09/M45

Topic: D.09. Tactile/Somatosensory Systems

Support: Boğaziçi University BAP project: 13XP8

Title: Effects of bicuculline and NMDA on the vibrotactile responses of cortical neurons in the rat SI cortex

Authors: *B. VARDAR, B. GÜÇLÜ;
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Abstract: We previously found that bicuculline (GABA_A receptor antagonist) and NMDA had contradictory, i.e. increasing and decreasing, effects on the average spike rates of neurons from the hindpaw representation in the rat SI cortex. We studied this in more detail by classifying neurons based on location (layers III, IV, V) and spike shape (regular-spiking (RS), fast-spiking (FS), and intrinsically bursting (IB)). We recorded single-unit spike activity during and after drug microinjection from 30 tactile neurons in 9 anesthetized Wistar albino rats. A combination electrode (5 injection barrels + 1 carbon-fiber electrode for recording) was used for applying aCSF (sham condition), 200 μM bicuculline, and 10 μM NMDA by a pneumatic microinjection pump (5 pulses of ~8 nL volume). After mapping the receptive field of each neuron, vibrotactile stimuli (bursts of 5-, 40-, and 250-Hz sinusoidal displacements; duration: 0.5 s; amplitude range: 33-158 μm) were applied on the glabrous skin of the hindpaw. Intracortical drug injection preceded the tactile stimulus. Average firing rates were calculated for the baseline before the stimulus (Rb) and for the entire stimulus duration (0.5 s) (Rd). 3-way ANOVAs (factors: stimulus frequency, cortical layer, drug vs. sham) showed significant main effects of vibrotactile frequency on Rd (p's = 0.001 and 0.046 for bicuculline and NMDA respectively). However, due to the large variation of responses, significant drug effects were not found in ANOVA. Therefore, we analyzed Rd based on paired comparisons between the drug and sham conditions at each frequency. Bicuculline significantly increased Rd at 5 and 250 Hz (paired t-test; p's =

0.010), but not at 40 Hz. On the other hand, NMDA significantly increased Rd only at 40 Hz (paired t-test; p = 0.020). For both drugs, Rd-Rb was mostly not affected, which shows that background firing rate somewhat also increased with drug application. For both drugs and at all frequencies, Rd was significantly increased in RS neurons (n=14). However, Rd of presumably inhibitory FS neurons (n=11) was not significantly affected by drug application. These results suggest that vibrotactile responses of the SI cortical neurons are influenced by complex excitatory and inhibitory interactions which are difficult to predict just by considering the established roles of the applied drugs.

Disclosures: **B. Vardar:** None. **B. Güçlü:** None.

Poster

065. Somatosensory Thalamocortical Processes

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 65.10/M46

Topic: D.09. Tactile/Somatosensory Systems

Support: Swiss National Science Foundation

European Research Council

Marie-Curie Re-integration Grant

Title: Whisking-related changes in neuronal firing and membrane potential dynamics in the somatosensory thalamus of awake mice

Authors: ***N. L. URBAIN**[1,2], P. SALIN[3], L. GENTET[4], C. PETERSEN[2];
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Abstract: In rodents, active somatosensory perception is particularly relevant through rhythmic and rapid sweeps of facial whiskers contacting objects in the surrounding environment. Faithful transmission of whisker-related sensory information from the periphery to the neocortex is thought to occur through the ventroposteromedial thalamic nucleus (VPM). On the other hand, neurons of the posterior group of the thalamus (Pom), the other major somatosensory thalamic nucleus, respond weakly to whisker stimuli. Our current knowledge of thalamic processing in the whisker sensorimotor system is largely based on recordings from anesthetised rats, but to uncover the functional relevance of these parallel sensory pathways, we carried out

electrophysiological recordings in awake animals during whisker-related behaviour. We therefore developed a technical approach to perform intra- and extracellular single-unit recordings in the mouse somatosensory thalamus coupled with local field potential recordings in primary somatosensory barrel cortex (S1-LFP) and video-tracking of whisker movements. Our results reveal strong correlations of both VPM and Pom neuronal activity with behavioural and cortical states. When mice enter quiet states, slow oscillations appear in S1-LFP and both VPM and Pom thalamic cells display abrupt reductions in spontaneous firing rates associated with membrane hyperpolarization. In contrast, while mice start whisking, thalamic cells shift to more depolarized potentials. During whisking, we find an increase in the rate of fast-rising EPSPs specifically in VPM neurons, contributing to drive increased spiking. We further find that a subset of VPM neurons code whisker position (phase) during the whisking cycle. In Pom cells, we do not observe an increase of fast-rising EPSPs related to free-whisking, but a significant increase in slow EPSPs (20-80% rise-time > 0.6 ms) during whisking compared with no-whisking waking state. Our data therefore demonstrate differential firing and subthreshold activity of VPM and Pom neurons during whisking behaviour.

Disclosures: **N.L. Urbain:** None. **P. Salin:** None. **L. Gentet:** None. **C. Petersen:** None.

Poster

065. Somatosensory Thalamocortical Processes

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 65.11/M47

Topic: D.09. Tactile/Somatosensory Systems

Title: The modulation of oral sensory information in the trigeminal and thalamic nuclei during superior laryngeal nerve stimulation

Authors: ***S. SAKAI**, T. SUZUKI, K. TSUJI, J. MAGARA, T. TSUJIMURA, M. INOUE; Niigata Univ., Niigata City/Niigata, Japan

Abstract: Introduction: The aim of the present study was to investigate the possible neuronal mechanisms for the modulation of oral sensory transmission during swallowing evoked by electrical stimulation of superior laryngeal nerve (SLN) in anesthetized rabbits. Materials and Methods: Experiments were carried out on rabbits anesthetized with urethane. Single unit responses evoked by the electrical stimulation of the inferior alveolar nerve (IAN) were recorded in either the trigeminal or thalamic nucleus. To evoke the swallowing reflex, SLN was stimulated and current intensity for SLN stimulation was set between 1 and 4 times of the threshold for evoking the swallowing reflex in 10 seconds. Activation of single neurons in either investigated

area was compared between with and without SLN stimulation. Recording sites in central regions were histologically identified. Results & Conclusion: 1. Responses of most neurons recorded in both the trigeminal and thalamic nuclei were inhibited during 2 or 4 times SLN stimulation. 2. Recording sites of the neurons were identified in the main sensory trigeminal nucleus and subnucleus oralis of the spinal trigeminal tract in the trigeminal nuclei and nucleus ventralis posteromedialis in thalamus. 3. Current results suggest that the transmission of oral sensation including ascending pathway is modulated during SLN-evoked swallowing. References: •Takako Fukuhara et al., 2011. Effects of electrical stimulation of the superior laryngeal nerve on the jaw-opening reflex. Brain Res. 1391: 44-53. •Aki Yamada et al., 2013. Effects of chewing and swallowing behavior on jaw opening reflex responses in freely feeding rabbits. Neurosci Lett. 535:73-7 •K.A. Olsson et al., 1986. Modulation of transmission in rostral trigeminal sensory nuclei during chewing. J Neurophysiol. 55(1): 56-75.

Disclosures: **S. Sakai:** None. **T. Suzuki:** None. **K. Tsuji:** None. **J. Magara:** None. **T. Tsujimura:** None. **M. Inoue:** None.

Poster

065. Somatosensory Thalamocortical Processes

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Topic: D.09. Tactile/Somatosensory Systems

Support: NIH Grant NS011862

Title: Representation of object properties and hand kinematics in somatosensory (S1) and posterior parietal cortex (PPC)

Authors: *E. P. GARDNER[1], J. L. BAKER[2], J. RYOU[2], J. CHEN[1];
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Abstract: To analyze the roles of PPC and S1 during skilled manual tasks, we trained monkeys to perform an instructed-delay bimanual prehension task, using visual cues to guide object selection. Five objects (2 spheres, 2 cylinders, 1 rectangular block) were arrayed symmetrically on a panel placed at shoulder height at the midline; icons on a computer screen represented their shape and/or location. In shape-cued trials, we displayed a single large red icon at the screen center whose shape matched that of the rewarded object. When the icon color changed from red to green, the animal had to reach, grasp, pull and hold a matching object. As there were two

spheres and cylinders, the animal could choose which hand to use; the rectangular block at the center was equally accessible to both hands. In shape+location-cued trials, five icons showed the shape and location of all 5 objects simultaneously. The rewarded object was colored red at trial start; its location on the right or left side of the screen indicated which hand to use. Kinematic contingencies were identical in both trial types. Motor planning of prehension thus required target selection and movement selection. To assess the role of task type during motor planning and performance, we recorded spike trains with microelectrode arrays implanted at bilaterally symmetric sites in the hand areas of PPC and SI. We propose that PPC activity reflects task goals to grasp and manipulate specific objects. SI responses are hypothesized to confirm or rebut the subject's expectation of object features, providing feedback for error correction. By interleaving blocks of shape-only and shape+location trials, we tested whether neuronal shape selectivity is enhanced when needed for correct performance, or whether parietal areas serve kinematic, not sensory cognitive function. Kinematic factors were reflected in both neural and behavioral activity. The majority of PPC cells responded to reach and grasp in both tasks and for all objects. Actions of the contralateral hand produced higher firing rates, but left and right hands evoked similar firing patterns in both hemispheres. In the shape task, the animal used the right hand to grasp the sphere and cylinder in >90% of trials, even though reward probability was equal for left and right objects. The rectangular block was grasped with the left hand, similar to the choice made in the location task. This suggests that the hand used reflected an individual preference for manipulating specific objects. Moreover, neural responses to each knob were similar in both tasks, suggesting that while the animal was cognizant of object form, neural activity in PPC was more tightly linked to hand actions than to object features.

Disclosures: **E.P. Gardner:** None. **J.L. Baker:** None. **J. Ryou:** None. **J. Chen:** None.

Poster

065. Somatosensory Thalamocortical Processes

Location: Hall A

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Topic: D.09. Tactile/Somatosensory Systems

Support: Wellcome Trust

Title: Selective activation of infragranular layers of vibrissal primary motor cortex inhibits vibrissal sensory pathway via corticofugal projections

Authors: *M. LOHSE, E. SADER, L. UPTON, E. MANN;
Dept. of Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: Sensorimotor integration is an important feature for optimising discrimination of the tactile world. We investigated how activation of vibrissal primary motor cortex (vM1) influences vibrissal sensory processing. We stimulated vM1 in anaesthetised mice using intra-cortical microstimulation, as well as optical stimulation of channelrhodopsin-2 (ChR2) expressed selectively in either vM1 layer V or layer VI. Whisker-evoked responses were recorded using both whole-cell patch-clamp recordings in vibrissal somatosensory cortex (vS1) and multi-unit recordings in somatosensory thalamus and vS1. We find that activation of vM1 inhibits whisker-evoked responses in both vibrissal sensory thalamus and cortex, with this inhibition decaying over a period of ~150 ms. We also show that activation of either vM1 layer V or layer VI is sufficient to drive activity in sensory thalamus, and inhibit subsequent vibrissal responses. Moreover, the inhibition of vibrissal responses in thalamus does not require activity in vS1. This suggests that sensory inhibition is initiated via direct corticofugal projections from vM1, rather than cortico-cortical connections. We propose that the inhibition comes about through reciprocal connections between thalamic nuclei (somatosensory and motor) and the thalamic reticular nucleus. This new sensorimotor phenomenon presents an opportunity for the nervous system to integrate information about the timing of motor commands and subsequent sensory stimulation.

Disclosures: **M. Lohse:** None. **E. Sader:** None. **L. Upton:** None. **E. Mann:** None.

Poster

065. Somatosensory Thalamocortical Processes

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Topic: D.09. Tactile/Somatosensory Systems

Support: BBSRC

Merck & Co

Title: Distinct mGlu2 and mGlu3 receptor functions in modulation of sensory responses and burst firing in the ventrobasal thalamus during vibrissa stimulation

Authors: ***T. E. SALT**[1], S. A. NEALE[2], C. S. COPELAND[1];
[1]UCL Inst. Ophthalmology, London, United Kingdom; [2]Neurexpert Ltd, London, United Kingdom

Abstract: Group II mGlu (mGlu2 and mGlu3) receptors modulate responses to vibrissa stimulation *in vivo* (Copeland et al 2012). Response patterns of thalamic neurones to sensory

stimulation, and in particular transitions from tonic to burst firing mode, are thought to be important in cognition and attention. We therefore investigated how activation or blockade of these mGlu receptors affects burst firing and tonic firing of ventrobasal thalamus (VB) neurones in response to vibrissae stimulation. Extracellular single neuron recordings were made *in vivo* with multibarrel electrodes in the VB of urethane-anaesthetized adult Wistar rats. Drugs were applied locally by iontophoresis. VB neurons were activated by vibrissa stimulation (10Hz, 1s duration trains), and evoked action potential spikes were isolated and timed. Responses to vibrissae stimulation consisted of spikes that were classified offline as occurring either in bursts or not in bursts (Copeland et al 2015). Application of the pan-Group II antagonist LY341495 caused a reduction in the number of action potentials evoked by sensory stimulation (to 81±5.0% of control; n=6) and at the same time decreased the proportion of burst firing (12±4.4% decrease). These effects were mimicked by the dual mGlu3 antagonist / mGlu2 agonist LY395756 (responses reduced to 59±4.8% of control, burst firing decrease 12±6.4%; n=6). By contrast, the pan-Group II mGlu agonist LY354740 increased sensory responses (177±24% of control; n=6) and increased the proportion of burst firing (16±3.8%). This action could be mimicked by co-application of LY395756 with the mGlu2 Positive Allosteric Modulator (PAM) LY487379: sensory responses increased to 154±7.2% and proportion of burst firing increased by 10±5.5% (n=6). These results provide the first direct demonstration of an mGlu3 receptor-mediated modulation of sensory responses that is activated during stimulation. Furthermore, this appears to operate in addition to, but greater than and opposing the effects mediated via mGlu2 receptors that we have described before (Copeland et al 2012). This shows that mGlu2 and mGlu3 receptors have distinct functions in sensory processing at the thalamic level and are placed to shift the balance between burst and non-burst (tonic) firing, underlining their importance in regulation of cognitive and attention processes. Copeland et al (2012), J Physiol 590: 937-951. Copeland et al (2015), Neuropharmacol 92: 16-24.

Disclosures: **T.E. Salt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Director of Neurexpert Ltd. **S.A. Neale:** None. **C.S. Copeland:** None.

Poster

065. Somatosensory Thalamocortical Processes

Location: Hall A

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Program#/Poster#: 65.15/N3

Topic: D.09. Tactile/Somatosensory Systems

Support: McKnight Foundation

NIH DA0171-88

Title: Layer- and cell-type segregation of thalamocortical input from POm in somatosensory cortex

Authors: *N. AUDETTE[1], M. MATSUSHITA[2], A. L. BARTH[1];
[1]Dept. Biol. Sci. and Ctr. for Neural Basis of Cognition, Carnegie Mellon Univ., Pittsburgh, PA;
[2]Carneie Mellon Univ., Pittsburgh, PA

Abstract: Thalamocortical inputs to the barrel cortex arise from the ventral-posterior nucleus (VPM), with dense axonal arbors in layer 4 and layer 5B, and from the posterior medial nucleus (POm) which projects to layer 1 and layer 5A. Prior work has shown that neurons in layer 2 show broad, multiwhisker subthreshold receptive fields that might be inherited from POm, since these thalamic neurons are also broadly tuned. In order to understand how different thalamic streams can influence firing of neocortical neurons, we used POm-targeted, viral-mediated channelrhodopsin expression to map POm inputs across cortical layers and cell types. Excitatory neurons in every layer receive direct, glutamatergic POm input, but the amplitude of this input is stratified by laminar location. Monosynaptic POm input was strongest in L5 neurons and then in L2 neurons. Interestingly, neurons in L2 receive significantly stronger input than neurons in L3, revealing a functional distinction between L2 and L3 neurons. Current clamp recordings show that POm engages deep and superficial neurons in two distinct patterns. Pyramidal neurons in L5 receive strong excitation rapidly followed by polysynaptic inhibition. In L2, optical activation of POm axons almost never elicited recurrent inhibition. To determine whether POm-triggered inhibition in layer 5 arises from direct activation of inhibitory neurons or recurrent activity in the neocortical circuit, we employed transgenic mice with different fluorescently labelled interneuron populations in combination with POm-targeted channelrhodopsin injections. Our recordings indicate that POm-initiated inhibition is likely to come from parvalbumin-expressing but not somatostatin-expressing interneurons. This widespread pattern of direct excitation confirms that PoM inputs may be an important source of the broad receptive fields observed in non-granular layers of the cortex.

Disclosures: **N. Audette:** None. **M. Matsushita:** None. **A.L. Barth:** None.

Poster

065. Somatosensory Thalamocortical Processes

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Topic: D.09. Tactile/Somatosensory Systems

Support: NIH R01NS084818

Title: Functional consequences of bilateral facial maps along the thalamocortical system

Authors: *V. TSYTSAREV[1], H. ARAKAWA[2], P. GASPAR[3], A. CHEDOTAL[3], R. S. ERZURUMLU[4];
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Abstract: Midline crossing defect of trigeminal principal sensory nucleus (PrV) axons leads to formation of bilateral whisker maps in the ventroposteromedial (VPM) nucleus of the thalamus and subsequently in the primary somatosensory or "barrel" cortex in mice. In the PrV, whisker-specific barrelette neurons are largely derived from rhombomere 3 (r3). Conditional mutation of the Robo3 receptor in r3-derived neurons results in midline crossing defects of the PrV trigeminothalamic axons. We used voltage-sensitive dye optical imaging methods (VSDi), and somatosensory behavioral tests in Krox20cre/Robo3lox/lox mice to delineate functional consequences of bifacial maps in the somatosensory thalamocortical system. We visualized neural activity in the barrel cortex of mice with bifacial representation of the whiskers in response to ipsi- and contralateral single whisker stimulation with VSDi using the voltage-sensitive dye RH-1691 and MiCAM-02 system. Change in fluorescence was calculated as ΔF/F (%) in the barrel field using the Brain Vision Analyzer. A glass pipette was aimed at the E2 whisker and air-puff stimulus of 25 ms duration was applied through a compressor coupled to the imaging system, so that the air could be puffed onto the whiskers and optical signals collected simultaneously. In control animals only contralateral whisker stimulation elicited VSDi signals in a given cortex, while ipsilateral stimulation didn't evoke any activity. In all examined mutant animals both ipsi- and contralateral whisker stimulation evoked VSD signals in the cortex. Unilateral transection of the infraorbital nerve further confirmed the presence of ipsi- and contralateral whisker responsiveness in the barrel cortex of mutant mice. We used a battery of sensorimotor tests to investigate the behavioral outcome of bifacial cortical maps. We tested for general whisker tactile sensation tests (horizontal approach, floating in water and sticky paper tests); whisker sensorimotor ability (gap crossing, bridge crossing); and tactile cognitive tests (shape-texture recognition); as well as detailed analysis of whisking patterns including whisking laterality and object localization. The mutant mice showed notable deficits in performance in all of the whisker-dependent sensory-motor tests. Particularly, the horizontal localization by whiskers was impaired. Thus bifacial maps along the thalamocortical system do not offer any functional advantages; instead they lead to impairments probably due to interference between the ipsi and contra whisker representations in the same thalamus or cortex. Supported by NIH R01NS084818

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Poster

065. Somatosensory Thalamocortical Processes

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 65.17/N5

Topic: D.09. Tactile/Somatosensory Systems

Title: Effect of long-range feedback connections from motor cortex and thalamus to somatosensory cortex

Authors: T. ZOLNIK[1], *R. N. SACHDEV[2], M. LARKUM[3];
[1]Neurocure Ctr. for Excellence, Humboldt Univ., Berlin, Germany; [2]Charite-Berlin, Berlin, Germany; [3]Neurocure Ctr. for Excellence, Humboldt University, Berlin, Berlin, Germany

Abstract: A signature of feedback and long-range connections between cortical areas is their projection to layer 1. The functional consequences of projections to this thin, cell sparse layer, filled primarily with the distal apical dendrites of pyramidal neurons and a small number of non-fast spiking interneurons are a mystery: Can these connections trigger a somatic response in layer 5? Are the connections to layer 1 uniform in their effect on postsynaptic targets? Here we compare the effects of two connections that target layer I -- motor cortex and POm (paralemniscal) thalamus -- in primary somatosensory cortex. We use an optogenetic approach to specifically activate POm or M1 axons in somatosensory cortex, while recording activity in L1 interneurons and L2/3 and L5 pyramidal neurons in brain slices. Though the POm and M1 axons have a differential distribution in layer 1 -- the M1 connections cover a larger portion of layer 1 -- both POm and M1 axons can activate all classes of neurons we studied here. But, POm axons activate the layer 1 network more strongly than M1 inputs do (POm, 18.5+ 4.4 mV; M1, 9.7+ 2.3 mV) and have a weaker effect on L2/3 pyramidal neurons than does the M1 projection (POm, 4.0+ 1.0 mV; M1, 7.5+ 2.7 mV). Surprisingly, L5 pyramidal neurons are activated to a similar extent by both inputs (POm, 9.8+ 1.9 mV; M1, 8.0+ 2.6 mV). A notable difference is that 20 Hz stimulus trains create different post-synaptic dynamics: the effect of M1 inputs decays more rapidly than the effect of POm inputs. Activation of these axons in layer 5 produces a faster, larger, and shorter latency EPSP response than does activation in L1. To examine the anatomical distribution of presynaptic neurons that target layer 1 and 5, we are using retrograde AAV6-fluorophor viral vectors. To examine the functional / differential consequences of activating M1 and POm inputs to S1, we are using the photo convertible calcium indicator CaMPARI (Fosque et al., 2015). The consequences of activating these inputs during a sensorimotor whisker dependent task are also under study.

Disclosures: **T. Zolnik:** None. **R.N. Sachdev:** None. **M. Larkum:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

Location: Hall A

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: PVA Research Foundation

VA CDA (1 IK2 RX001123-01A2)

Title: Therapeutic window for correcting dendritic spine dysgenesis in SCI induced neuropathic pain

Authors: *A. M. TAN, S. LIU, M. HILL, P. ZHAO, S. G. WAXMAN;
Neurol., Yale University/VA Connecticut Healthcare Syst., West Haven, CT

Abstract: The primary objective of our work is to reveal mechanisms that contribute to neuropathic pain after spinal cord injury (SCI). Dendritic spines are micron-sized postsynaptic structures that represent modifiable sites of synaptic contact and contribute to neuronal physiology. Importantly, dendritic spines provide crucial insights into how neural networks form and retain function. Because dendritic spines are located on dorsal horn neurons within the spinal cord nociceptive system, dendritic spine remodeling provides a unique perspective to understand and address sensory dysfunction after SCI. Over fifty percent of patients with SCI experience neuropathic pain that is refractory to current medical treatment. Our previous work has shown a structure-function relationship between abnormal spine morphologies and neuropathic pain by demonstrating that acute pharmacological inhibition of Rac1-regulated dendritic spine remodeling on nociceptive dorsal horn neurons significantly attenuates electrophysiological and behavioral symptoms of neuropathic pain. In the present study, to identify a therapeutic window for correcting dendritic spine dysgenesis in SCI-induced neuropathic pain, we addressed two questions: First, does anti-Rac1 drug treatment early after SCI (<24hrs) prevent the progression of abnormal dendritic spine plasticity and the development of SCI-induced neuropathic pain? Second, does cessation/withdrawal of anti-Rac1 drug treatment allow relapse of SCI-induced abnormal dendritic spine morphology and neuropathic pain? Our findings demonstrate that Rac1 inhibition early after SCI has little to no efficacy in preventing the development of SCI-induced neuropathic pain, and that abnormal dendritic spine and pain pathologies relapse following cessation of anti-Rac1 drug treatment. Overall, our findings suggest the need for a more durable therapeutic strategy. To this end, we are developing a gene therapy approach to target dendritic

spine remodeling associated with neuropathic pain and other neuronal-hyperexcitability diseases after SCI.

Disclosures: **A.M. Tan:** None. **S. Liu:** None. **M. Hill:** None. **P. Zhao:** None. **S.G. Waxman:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 66.02/N7

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Awake behaving electrophysiological correlates of weakness and spasticity after cervical hemi-contusion in the rat

Authors: ***P. D. GANZER**[1,2], E. MEYERS[1,2], R. L. RENNAKER, II[1,3,2], M. P. KILGARD[3,2]; [1]Bioengineering, Univ. of Texas At Dallas, Richardson, TX; [2]Texas Biomed. Device Ctr., [3]Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas, Richardson, TX

Abstract: Cervical spinal cord injury (cSCI) is a major cause of disability, in many cases leading to long lasting impairments in forelimb function and quality of life. cSCI can interrupt descending motor control, promote aberrant sprouting of spinal afferent fibers and modify spinal excitability, which may all contribute to forelimb weakness and spasticity. In this study, we model hemi-contusion cSCI in the rat and assess its effects on forelimb strength and muscular dynamics (using awake behaving biceps and triceps EMG recordings). Prior to injury, animals: 1) were trained to proficiency on the isometric pull task, which quantitatively measures multiple parameters of forelimb strength; and 2) were tested for baseline paw withdrawal to a thermal stimulus using the Hargreaves method. After cSCI, testing was performed each week for 4 weeks. In a subset of animals, we also recorded biceps and triceps EMG during these assessments. Our results show classic attributes of spasticity following cSCI: weakness, increases in motor variability and withdrawal hyper-reflexia. We then used factor analysis to investigate the pre-motor drives associated with EMG activity across assessments before and after cSCI. Our results highlight the electrophysiological dynamics associated with interrupted descending motor control, aberrant spinal sprouting and modified spinal excitability reported previously after SCI and other types of upper motor neuron insults.

Disclosures: **P.D. Ganzer:** None. **E. Meyers:** None. **R.L. Rennaker:** None. **M.P. Kilgard:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

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VA Grant I01RX000815

VA Grant I01RX001807

Craig H. Neilsen Foundation Grant 261299

Title: Decoupling of hand and arm movements after spinal cord injury

Authors: *F. J. CALABRO[1,2], M. A. PEREZ[2,3];
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Abstract: We have previously shown that spinal cord injuries impair the ability to execute bilateral reach-to-grasp movements, resulting in pronounced movement time delays of the stronger arm. The extent to which this reflects a deficit in interlimb coordination and/or a compensatory strategy to synchronize movements with the more impaired arm remains unknown. To address this issue, we studied bilateral self-paced and ballistic reach-to-grasp movements of a small (1 cm) and large (7.5 cm) cylinder in 16 individuals with incomplete cervical SCI and 20 uninjured controls. We found that SCI subjects exhibited reduced trial-by-trial correlations (decoupling) in the magnitude and in the time to reach maximum hand aperture compared with controls, regardless of object size and movement speed tested. However, there were no differences in the correlation between the magnitude and the time to reach peak arm velocity between SCI subjects and controls. In addition, SCI subjects showed a decreased synchronization during hand maximum aperture (time at which each hand reached maximum aperture) and grasping (time at which each hand grasped the object with the index finger and thumb) compared with controls in all conditions tested, suggesting that it is less likely that bilateral movement time delays were related to a compensatory strategy to synchronize movements with the more impaired arm. Thus, our results demonstrate that following cervical

SCI decoupling of the arms occurs at distinct phases during the reach-to-grasp movement, being present during grasping but not during arm acceleration. We suggest that this may reflect impaired interlimb coordination during grasping, providing an important target for rehabilitative interventions.

Disclosures: F.J. Calabro: None. **M.A. Perez:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

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VA Grant I01RX001807

Craig H. Neilsen Foundation Grant 261299

Title: Altered suppression of corticospinal drive prior to an upcoming action after spinal cord injury

Authors: *P. FEDERICO[1,2], M. A. PEREZ[1,2];
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[2]Dept. of Physical Med. and Rehabil., Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The ability to stop voluntary movement at a desired time is impaired in a number of individuals with incomplete spinal cord injury (SCI). The extent to which the corticospinal system is involved in this impairment after SCI remains unknown. Here, we used a GO and NOGO paradigm to test transmission in cortical and subcortical pathways prior to the execution and suppression of an upcoming voluntary command in humans with chronic incomplete cervical SCI and uninjured controls. Using noninvasive cortical and cervicomedullary stimulation we tested motor evoked potentials (MEPs) and short-intracortical inhibition (SICI) in the first dorsal interosseuous muscle during 2-5% of maximal voluntary contraction 60.1±31.4 ms after an

imperative signal. Index finger reaction time was shorter in controls (263.5±33.5 ms) compared with SCI subjects (292.9± 34.1 ms) at matched levels of background electromyographic activity. We found in GO trials that the size of cortically evoked MEPs increased and SICI decreased compared to baseline in both groups of subjects. In NOGO trials, MEP size decreased in controls (by 37.1±16.6%) but remained similar to baseline in subjects with SCI (decrease by 11.9±28.2%). Cervicomedullary MEP size increased compared to baseline in control but not in SCI subjects in GO trials, while it remained similar to baseline in both groups in NONGO trials. These findings suggest an impaired ability to suppress corticospinal drive prior to an upcoming command to stop a movement after SCI, likely related to deficits at the cortical level. Thus, feedforward cortical mechanisms might play a role in movement deficits after human SCI.

Disclosures: P. Federico: None. **M.A. Perez:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

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VA Grant I01RX001807

Craig H. Neilsen Foundation Grant 261299

Title: Strengthening corticospinal synaptic transmission in a lower-limb muscle

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Abstract: Repeated pairs of presynaptic and postsynaptic volleys arriving within a precise time window at the spinal cord increase the efficacy of corticospinal transmission in upper-limb

muscles (Taylor and Martin, 2009; Bunday and Perez, 2012). Here, we examined whether spike-timing-dependent plasticity can be induced in synapses targeting lower limb muscles. Transcranial magnetic stimulation over the leg motor cortex activated corticospinal neurons producing presynaptic action potentials, whereas electrical stimulation of the common peroneal nerve elicited antidromic action potentials to depolarize tibialis anterior (TA) motoneurones. Motor evoked potentials (MEPs) in the TA muscle were measured before and after 200 pairs of stimuli delivered at 0.1 Hz in control subjects. Latencies of the M-wave (4.9±0.1 ms), F-wave (35.2±3.1ms), and MEP (30.7±2.2 ms) in the TA muscle were determined for each subject to calculate peripheral [PCT=(F-wave latency - M-wave latency) * 0.5] and central conduction times [CCT=MEP latency - (PCT + M-wave latency)]. Conduction times (CCT=10.7±1.6 ms; PCT=15.2±1.4 ms) were used to time the arrival of presynaptic volleys ~2 ms prior to postsynaptic volleys. We found that the size of TA MEPs increased by ~70% above the MEP baseline immediately after paired stimulation and remained increased for up to 30 minutes after the last pair of stimuli. The increase in MEP size was observed for up to 50 minutes in a subset of subjects. These findings demonstrate that spike-timing-dependent like plasticity can be induced in corticospinal projections targeting lower-limb muscles, which might open new targets for enhancing voluntary motor output in individuals with motor disorders.

Disclosures: **M. Urbin:** None. **M.A. Perez:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

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Craig H. Neilsen Foundation Grant 261299

Title: Subcortical contribution to bimanual force coupling after spinal cord injury

Authors: J. LONG[1,2], T. TAZOE[1,2], *M. OUDEGA[1,2], M. A. PEREZ[1,2];
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[2]Dept. of Physical Med. and Rehabil., Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Previous evidence showed that cortical mechanisms contribute to the coupling of bimanual isometric forces in intact humans. Here, we examined the contribution of subcortical pathways to bimanual force coupling by testing individuals with and without subcortical damage due to incomplete cervical spinal cord injury (SCI). Subjects were instructed to perform unilateral and bilateral ballistic symmetric and asymmetric increasing levels of isometric index finger abduction (5%, 10%, and 30% of maximal voluntary contraction) when visual feedback of left and right forces were displayed with a single 2D cursor (Single-task) or two independent cursors (Dual-task). Force and electromyographic (EMG) activity in the first dorsal interosseous muscle were measured in all conditions. We demonstrate in uninjured controls that force and mean rectified EMG activity produced by the left and right hand were positively correlated in the Dual-task (both hands produced a similar motor output regardless of the asymmetry between targets) but not the Single-task (each hand produced a different motor output following the asymmetry of targets), suggesting that in controls the ability to engage the hands was modified by changes in the task demands imposed by the visual feedback. In contrast, SCI subjects showed that force and mean rectified EMG activity produced by both hands was positively correlated in the Dual-task and Single-task, suggesting an impaired ability to exert different levels of asymmetric forces between hands. Our results together indicate that subcortical pathways contribute to the coupling of independent bimanual isometric forces in humans.

Disclosures: J. Long: None. **T. Tazoe:** None. **M. Oudega:** None. **M.A. Perez:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

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Craig H. Neilsen Foundation Grant 261299

Title: Late TMS-induced I-waves detect loss of voluntary motor output after spinal cord injury

Authors: *J. CIRILLO[1,2,3], M. A. PEREZ[3,4];
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Abstract: Using transcranial magnetic stimulation (TMS) we showed that late TMS-induced indirect (I) waves are critical for recruitment of spinal motoneurones in humans with anatomically incomplete spinal cord injury (SCI; Cirillo et al., 2015). Here, we examined whether late TMS-induced I-waves are sensitive to detect changes in voluntary motor output after injury. A single-pulse TMS was used over the hand motor cortex to elicit motor evoked potential (MEPs) in an intrinsic finger muscle during small levels of isometric voluntary contraction in uninjured controls and in individuals with incomplete chronic cervical SCI. During testing, the TMS coil was oriented in the latero-medial (LM), posterior-anterior (PA), or anterior-posterior (AP) direction to preferentially activate corticospinal axons directly, and early or late I-waves, respectively. In controls, we found that MEP latency was delayed by 1.8±0.5 ms with PA and by 3.3±1.1 ms with AP compared with the LM. In SCI subjects, we found two groups of individuals; one with similar latency differences as controls (LM-PA=1.6±0.6 ms and LM-AP=3.5±1.1 ms) and another where latency difference was not different across conditions (LM-PA=1.5±0.9 ms and LM-AP=1.6±1.3 ms). SCI subjects whose delay for LM-AP coil orientation was not different with LM-PA exerted significantly less maximal force and electromyographic (EMG) activity than SCI subjects with similar latency differences as controls. Notably, a positive correlation was found between latency differences in the LM-AP direction and force and EMG outcomes in all SCI subjects. Our findings indicate that late TMS-induced I-waves are a sensitive measure to detect loss of voluntary motor output after human incomplete SCI.

Disclosures: J. Cirillo: None. **M.A. Perez:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

Location: Hall A

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Program#/Poster#: 66.08/N13

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Control of diaphragm activity after SCI in the absence of supraspinal input: the contribution of interneurons

Authors: *J. M. CREGG, L. T. LANDMESSER, J. SILVER;
Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: Respiratory drive is relayed to phrenic motor neurons via bulbospinal projections. Spinal cord injury at or above cervical level 4 disrupts these descending projections and causes permanent loss of diaphragm function. In this study we examined whether cervical spinal cord circuitry--circuitry that retains local connectivity after high cervical spinal cord injury--can be mobilized to control the diaphragm in the absence of descending input from bulbospinal premotor nuclei. Using an *in situ* model of cervical spinal cord injury we demonstrate that in the absence of supraspinal input, tonic inhibitory synaptic transmission suppresses phrenic motor output. Pharmacological blockade of GABAergic/glycinergic transmission relieves inhibitory synaptic input to phrenic motor neurons, and furthermore, uncovers an excitatory propriospinal circuit capable of driving synchronous right/left phrenic motor output. Under conditions of disinhibition, we find that we can precisely control phrenic motor output by optogenetic stimulation of excitatory interneurons at defined frequencies. These results identify a discrete microcircuit in the cervical spinal cord that can coordinate right/left phrenic motor output in the absence of descending command from bulbospinal premotor nuclei. We further show that this circuit can be mobilized to promote hemidiaphragm function after lateral C2 hemisection *in vivo*. Thus, taken together, we demonstrate that the rich functional diversity of cervical phrenic interneurons can be exploited to allow function of an otherwise paralyzed muscle required for sustaining life.

Disclosures: J.M. Cregg: None. **L.T. Landmesser:** None. **J. Silver:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH-NS 025713

Case Western Reserve University Council to Advance Human Health

Unite to Fight Paralysis

Spinal Cord Injury Sucks

Title: Modulation of the proteoglycan receptor PTPσ induces neuronal protease release to overcome Chondroitin Sulfate Proteoglycan inhibition of axon regeneration

Authors: *A. TRAN[1], B. T. LANG[2], J. SILVER[1];
[1]Neurosciences, Case Western Reserve Univ., Cleveland, OH; [2]Athersys, Cleveland, OH

Abstract: Following spinal cord injury, newly reformed growth cones of regenerating axons are halted by the extracellular matrix (ECM) of the glial scar. One ECM component in particular, chondroitin sulfate proteoglycans (CSPGs), actively prevent regeneration and sprouting through increased expression in both the lesion scar and perineuronal nets surrounding deafferented neurons. Protein Tyrosine Phosphatase-Sigma (PTPσ) has been identified as a receptor for CSPGs that is sufficient to signal axonal growth inhibition. Further, manipulation of PTPσ with the novel Intracellular Sigma Peptide (ISP), unlocks axonal plasticity within CSPG rich regions through a currently unknown molecular mechanism. We previously demonstrated that ISP treatment enhances recovery of sensorimotor, locomotor, and urinary function following severe spinal cord injury via unprecedented serotonergic sprouting (Lang et al. 2015). We now report that modulation of PTPσ, both *in vivo* and *in vitro*, increases the release of protease(s) from neurons that digest inhibitory components of the ECM, such as aggrecan. Using gel zymography, western blots, and *in vitro* assays, we were able to demonstrate that ISP induced protease digestion of aggrecan. Thus, we hypothesize that one of the critical downstream events underlying the failure of axon regeneration is a lack of cell growth-permitting protease release within CSPG rich regions. Further understanding of the mechanisms underlying CSPG-PTPσ signaling will further elucidate how axon regeneration is impaired following spinal cord injury.

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Poster

066. Spinal Cord Injury: Neuroplasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: CONACYT scholarship 337092 (E.Ch.)

Promep-SEP grant PTC-244 (C.A.P.)

Title: Effect of spinal cord injury on the expression of c-Fos in the cerebellum of male rats

Authors: *C. A. PEREZ-ESTUDILLO[1,2], E. CHANG-MOYA[2], M. L. LOPEZ-MERAZ[2], C. MORGADO-VALLE[2], L. BELTRAN-PARRAZAL[2], A. J. MARTINEZ-CHACON[3], G. A. CORIA-AVILA[2], J. MANZO[2];
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Abstract: The cerebellum participates in the regulation of fine movements, sexual responses and motor learning, including balance. Therefore it is a structure that integrates information arriving from the forebrain, but also from the spinal cord. Any change in the physiology of those structures is likely to modify the neural activity within the cerebellum. Thus, in the present study we assessed the expression of c-Fos in the cerebellum of male rats after 6 or 14 h of a spinal cord injury. Adult Wistar males were used. In a first experimental design they were divided into three groups: 1) intact, 2) sham and 3) 6 h after spinal cord injury. In a second experiment they were divided into: 1) intact, 2) sham and 3) 24 h after spinal cord injury. The lesion was performed by using a drop-weight technique. Thus, a 10 g weight was dropped from a height of 15 cm through a guide tube. The expression of c-Fos was then assessed with immunocitochemistry. The number of positive cells to c-Fos on the cerebellar vermis was analyzed through a Generalized Linear Model. The results of the first experiment showed that 6 h after the lesion there was a significant increase ($p< 0.01$) on the c-Fos expression of the granule cell layer of lobules 1, 5, 7, 9 and 10, as well as on the Purkinje layer of lobules 1, 7 and 9. For the second experiment, we observed that 24 h after the lesion there was an increase of c-Fos on the granule cell layer of lobules 3, 4 and on the fastigial deep nucleus (in comparison with both intact and sham rats). These results suggest that there are specific responses in a timed manner within the cerebellum after loss of spinal input. Such responses may be associated with biochemical processes that occurred during the primary damage (after 6 h), and with the secondary damage (after 24 h). Additionally, these results show that the cerebellum activates “motor” and “non-motor” lobules after the occurrence of the spinal cord injury.

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Poster

066. Spinal Cord Injury: Neuroplasticity

Location: Hall A

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Provincial Key Developing Disciplines

Hebei Province,China

Title: The 5-HT1 receptors are involved in the increased ability of AADC cells to produce 5-HT following spinal cord injury

Authors: *L. REN[1], K. ZHANG[2], L. CHEN[2], H. HULTBORN[3];
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Abstract: Spasticity is a common disorder following spinal cord injury (SCI). Several mechanisms may contribute to the development of spasticity, but one important mechanism seems to be the return of persistent inward current in motor neurons which is controlled by their monoaminergic innervation. Following a spinal cord transection the descending monoaminergic systems are removed. However, spinal motor neurons become supersensitive to monoamines (receptor up-regulation) and spinal aromatic L-amino acid decarboxylase (AADC) cells could change their phenotype to be able to produce serotonin(5-HT) from 5-hydroxytryptophan after SCI. One possible mechanism for the increased ability of AADC cells to produce 5-HT following SCI might be the release of their repression by the raphe-spinal serotonergic innervation. We had previously found that 5-HT1B receptors could suppress 5-HT expression in the AADC cells, when a 5-HT1B receptor agonist (CP94253) was subcutaneously (s.c.) injected daily (0.1 mg/kg) for 8 consecutive days to chronic spinal animals (spinalized at S2) (Wienecke J et al ,J Neurosci. 2014, 34:11984-2000). We now investigate whether the other 5-HT1 receptor subgroups may also exert an inhibition of the AADC-enzyme e.g., 5-HT1A, 5-HT1D and 5-HT1F. We also investigated whether higher doses of the 5-HT1B agonist (CP94253) would be more effective than in our previous experiments. To investigate these hypotheses, we adopted the chronic S2 spinal cord transection model. A total of 42 Wistar rats were used.6 normal and 6 spinalized rats were used for AADC, 5-HT, 5-HT1A, 5-HT1B, 5-HT1D and 5-HT1F immunostaining. 24 spinalized rats were divided into 4 groups (Group 1A/1B/1D/1F) and each group(6 rats) received daily s.c. injection for 8 days with their agonist (5-HT1A agonist, (R(+)-8-OH-DPAT,0.3 mg/kg;5-HT1B agonist, CP94253, 0.3 mg/kg;, 5-HT1Dagonist, PNU-142633, 0.3 mg/kg; 5-HT1F agonist, LY-344864, 1 mg/kg; 6 normal rats were injected saline as control). 5-HT1A, 5-HT1B, 5-HT1D - but not 5-HT1F - immunolabeling were all found in AADC cells in the spinal cord of normal rats. Only 5-HT1B was expressed in AADC cells after SCI, 5-HT1B agonist can inhibit 5-HT production, but 5-HT1A /5-HT1D /5-HT1F agonist and saline had no

impact. 5-HT1B agonist (0.3 mg/kg) can inhibited more than 80% of AADC cells to produce 5-HT.In summary, our results demonstrate that 5-HT1B receptor play an important role in the enhanced ability of AADC cells to produce 5-HT after SCI.

Disclosures: **L. Ren:** None. **K. Zhang:** None. **L. Chen:** None. **H. Hultborn:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Canadian Institutes of Health Research Operating Grant

Title: Opening a window of delayed spinal plasticity following ischemic brain injury

Authors: *A. M. WIERSMA[1,2], I. R. WINSHIP[1];
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Abstract: Recent studies suggest that significant adaptive plasticity occurs in the spinal cord after stroke. Our recent work suggests that this delayed spinal plasticity has a finite temporal window that closes concurrent with a plateau in spontaneous recovery, suggesting that reopening this window may improve recovery. Here, we assessed if targeted pharmacotherapy administered to the spinal cord during the chronic phase of stroke could restart spinal plasticity and augment functional recovery. To augment spinal plasticity, adult male Sprague-Dawley rats received intraspinal injections of Chondroitinase ABC (ChABC) into the cervical spinal cord one month after a photothombotic stroke lesioning the forelimb sensorimotor cortex. Prior to stroke, rats were trained on the Montoya Staircase task or the single pellet reaching task, behavioural tests commonly used to assess skilled forelimb function after brain injury. Forelimb use preference was also determined using the cylinder task. Reaching performance was assessed at baseline and between 3 and 28 days post-stroke. Rats with persistent reaching deficits at 28 days were assigned to treatment groups that received 1 μL unilateral intraspinal injections at C5 and C6 of the cervical cord (i.e. into the spinal grey matter receiving corticospinal input from the stroke affected cortex) of ChABC (10 U/ml) or penicillinase (10 μg/ml, the control injection for ChABC). After injection, rats were divided into groups that received no rehabilitation or task-specific reaching rehabilitation of varying intensity. Reaching testing was repeated for 30 days after spinal injection. Our data show that ChABC injection improves skilled reaching after

delayed intraspinal administration, even without reaching rehabilitation. In addition, we found that ChABC can potentiate the therapeutic effect of task-specific (skilled reaching) rehabilitation initiated even one month after cortical stroke. Following final behavioural testing, anterograde neuronal tract tracers were injected near the sight of ischemic injury to assess changes in spinal cord connectivity from the spared cortex adjacent the infarct. We found that ChABC spinal injection significantly increased the number and distribution of ipsilesional corticospinal tract axons innervating stroke affected spinal grey matter. These experiments suggest that functional connectivity between the cervical spinal cord and sensory-motor cortex can be augmented long after cortical ischemic damage has occurred, and that reopening the window for spinal plasticity improves spontaneous and rehabilitation-induced recovery from cortical stroke.

Disclosures: A.M. Wiersma: None. **I.R. Winship:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH NINDS NS047567

Title: The firing properties of deep dorsal horn neurons following acute spinal cord injury during administration of agonists for NMDA and 5HT1 receptors

Authors: *T. THAWEERATTANASINP[1], C. J. HECKMAN[2], V. M. TYSSELING[3]; [1]Physiol., Northwestern University, Feinberg Sch. of Medici, Chicago, IL; [2]Physiology, Physical Med. and Rehabilitation, Physical Therapy and Human Movement Sci., [3]Physiology, Physical Therapy and Human Movement Sci., Northwestern University, Feinberg Sch. of Med., Chicago, IL

Abstract: Spinal cord injury (SCI) induces the imbalance of excitatory and inhibitory input to the spinal motoneurons. SCI enhances the excitatory input over the inhibitory one, thus leading to hyperreflexia and muscle spasms. This over-excitation could be attributed to the loss of tonic serotonin (5-hydroxytryptamine; 5-HT) inhibition of the excitatory deep dorsal horn (DDH) neurons. The SCI-induced disinhibition of the DDH neurons could increase their firing responses by showing bursting responses or increased tonic firing. Thus, the disinhibited DDH neurons potentially provide an exaggerated excitatory drive to the spinal motorneurons. Surprisingly, activation of 5-HT1B/1D receptors can reduce muscle spasms without affecting the intrinsic

properties of spinal motoneurons, shown by studies in chronic SCI rodents and humans. Moreover, activation of N-methyl-D-aspartate (NMDA) receptors can induce the potentiation of excitatory postsynaptic potentials. Thus, NMDA receptors could be also involved in exaggerating the excitatory drive to the spinal motoneurons after SCI. However, it remains unknown how the activation of 5-HT1B/1D and NMDA receptors would affect the responses of the DDH neurons after SCI. Therefore, we characterize the firing properties of the DDH neurons during the acute stage of complete SCI in bath applications of NMDA and the selective 5-HT1B/1D receptor agonist zolmitriptan. Adult mice (Mus musculus) received a complete spinal transection during the *in vitro* sacral cord preparation for the acute SCI model. Extracellular recordings of firing properties were made from the DDH neurons located in the spinal lamina III-V, which received synaptic activation via dorsal root stimulation at four stimulus intensities. We found three types of the DDH neuronal responses upon dorsal root stimulation: simple, bursting, and tonic responses. In response to increasing stimulus intensities in controls, the majority of the recorded DDH neurons showed the increasing number of their responsive spikes, faster first-spike latency, and larger field potentials. Compared with the controls, NMDA increased both the number of responsive spikes and tonic firing, whereas zolmitriptan decreased both of them and also delayed the first-spike latency. In case of the DDH neurons with bursting responses, they also showed an increase in their bursting duration in response to increasing stimulus intensities. Compared with the controls, NMDA increased the bursting duration, while zolmitriptan decreased it. Together, these results suggest the facilitative effect of NMDA and inhibitive effect of zolmitriptan on the firing properties of the DDH neurons after acute SCI.

Disclosures: **T. Thaweerattanasinp:** None. **C.J. Heckman:** None. **V.M. Tysseling:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 66.14/N19

Topic: D.10. Spinal Cord Injury and Plasticity

Support: RFBR Grant 13-04-00059

Title: Changes of calbindin-D28k immunoreactive neurons in the thoracic spinal cord after space flight

Authors: ***P. M. MASLYUKOV**, V. PORSEVA, A. STRELKOV, V. SHILKIN;
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Abstract: Calcium-binding proteins are involved in numerous functions, including cell signaling, calcium uptake and transport, cell motility and intracellular calcium acceptance. Calbindin-D28k (CB) is a calcium-binding protein located in separate groups of neurons including spinal cord. In studies of the spinal cord under microgravity condition, the most attention was focused on lumbar motoneurons. There are no data about structure and functions of thoracic motoneurons and interneurons after space flight. The aim of the work was to analyze changes in the location and morphological characteristics of CB-immunoreactive neurons of the thoracic spinal cord of C57BL/6N male mice after completion of a 30-day space flight on board the BION-M1 biosatellite (Russia, 2013). CB-positive neurons were identified using immunohistochemistry with subsequent fluorescent microscopy. Space flight induced multidirectional changes of the number and morphological parameters of CB-positive neurons. The number of IR neurons increased in laminae I (from 10 to 17 neurons per section), II (from 42 to 67 cells per section) and IX (from 2 neurons per segment to 2 neurons per section), but CB disappeared in neurons of laminae VIII. Weightlessness did not affect the number of CB-immunoreactive neurons in lamina III-V and VII including preganglionic sympathetic neurons. The cross-sectional area of CB-immunoreactive neurons decreased in lamina II and VII (group of partition cells) and increased in laminae III-V and IX. After a space flight, few very large neurons with long dendrites appeared in lamina IV. The results obtained give evidence about substantial changes in calcium buffer system and imbalance of different groups of CB-immunoreactive neurons due to reduction of afferent information under microgravity.

Disclosures: P.M. Maslyukov: None. **V. Porseva:** None. **A. Strelkov:** None. **V. Shilkin:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 66.15/N20

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Dentification of neuroregeneration promoting and inhibiting proteins present in cellular cultures of the olfactory ensheathing cell (oecs)

Authors: *M. Y. SANCHEZ, III[1,2], R. M. GOMEZ B[7], J. J. NIÑO[8], R. H. BUSTOS[3], M. A. DOMINGUEZ[4], D. VARGAS[5], M. F. QUIROZ-P[6];
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Enfermería y Rehabilitación, [5]Univ. de Medicina, [6]Facultad de psicología, Univ. de la Sabana, Chía, Colombia; [7]Fundación para el desarrollo de la neutro-regeneración en Colombia, Bogota - Cundinamarca, Colombia; [8]Fundación para el desarrollo de la neutro-regeneración en Colombia, Bogota- Cundinamarca, Colombia

Abstract: The olfactory ensheathing cells (OECS) have been of great interest since they are promotors of the regeneration of the central nervous system (CNS) due to their inherent capacity in the olfactory system to support the continuous regeneration of olfactory neurons. These cells secrete proteins that help the occurrence of neurogeneration processes. Previously the mechanisms of cellular action in a spinal cord injury has been studied, specifically de OECS in animal models regarding the favoring of the recovery of the locomotive function. Even do, in previous studies performed by us have demonstrated the effect of these factors *in vivo*; it was necessary to quantify them *in vitro* so as to understand on a cellular level the process of regeneration. It is this way, that this research had as a purpose to: Identify the presence and expression of stimulating factors such as: aFGF, NT-4/5 and inhibitors such as: NOGO-A and MAG of neuroregeneration in cellular cultures and conditioned media of the olfactory ensheathing cells (OECS) using nanosensor technology as the biosensors that allow to determine in the real time the concentration of the secretions of these factors with a minimum sample, without pretreatment, free of markers of revealing agents facilitating this way the effective evaluation of the presence and expression of promoting and inhibiting factors for the neuroregeneration of the OECS. Results obtained, in the cellular culture phase showed a purification of the measured cells by flow cytometry. Posteriorly it was established the detection and quantification methodology of the promoting and inhibiting proteins aFGF, NT-4/5 and inhibitors NOGO-A and MAG following the interaction kinetics using SPR (Surface Plasmon Resonance). Data obtained with the optical biosensor, show detection results for the promoting factors in concentrations in a nm scale significant for the different cellular densities and sampling time of the culture media of the olfactory ensheathing cells. Standard curves were drawn to calculate the concentrations of the proteins according to the ICH harmonization guides. Finally the methodology was validated using and immunoassay (ELISA).

Disclosures: **M.Y. Sanchez:** 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.; Universidad Nacional de Colombia . Facultad de Medicina, Universidad de la Sabana. **R.M. Gomez B:** 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.; Fundación para el desarrollo de la neutro-regeneración en Colombia. **J.J. Niño:** 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.; Fundación para el desarrollo de la neutro-regeneración en Colombia.

R.H. Bustos: 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. Universidad de la Sabana. Facultad de Medicina. **M.A. Dominguez:** 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.; Universidad de la Sabana, facultad de Enfermería y rehabilitación. **D. Vargas:** 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.;Universidad de la Sabana.facultad de Medicina. **M.F. Quiroz-P:** 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. Universidad de la Sabana. facultad de Psicología.

Poster

066. Spinal Cord Injury: Neuroplasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 66.16/N21

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NS041548

HD058412

Title: Loss of descending serotonergic fibers transforms how GABA regulates nociceptive systems within the spinal cord: Role of KCC2

Authors: *Y.-J. HUANG[1], K. H. LEE[2], J. W. GRAU[1];
[1]Neurosci., Texas A&M Univ., College Station, TX; [2]Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA

Abstract: GABA is the chief inhibitory neurotransmitter in the mammalian central nervous system. Given this, disrupting GABA transmission within the spinal cord with the GABA-A antagonist bicuculline should enhance neural excitability and promote the development of nociceptive sensitization. We have shown, however, that bicuculline paradoxically attenuates behavioral and cellular indices of central sensitization in spinally transected rats. Rats that have undergone a spinal transection at the second thoracic vertebrae (T2) exhibit enhanced mechanical

reactivity (EMR), and ERK activation within the dorsal horn, when a hind paw is treated with capsaicin. Intrathecal (i.t.) bicuculline blocks both of these effects. We proposed that spinal injury alters GABA signaling because it down-regulates KCC2, which would cause intracellular Cl- concentrations to rise and lead to GABA having an excitatory (depolarizing) effect. Supporting this, we have shown using western blotting that spinal injury reduces membrane bound KCC2 (relative to the cytoplasmic fraction) and that pharmacologically inhibiting KCC2 (via i.t. DIOA) emulates the effect of spinal injury in intact rats. Conversely, pharmacologically lowering intracellular Cl, via i.t. bumetanide (a NKCC1 inhibitor), in transected rats reverses the effect of bicuculline. We hypothesized that spinal injury alters GABA function because it disrupts descending serotonergic (5HT) pathways that regulate nociceptive transmission. If this is true, administration of a 5HT agonist should reinstate GABAergic inhibition after spinal injury and reverse the effect of bicuculline. Spinally transected rats were given a 5HT1A agonist (DPAT) prior to bicuculline and peripheral capsaicin. In the absence of DPAT, bicuculline attenuated nociceptive sensitization. In DPAT treated transected rats, bicuculline had the opposite effect.

Disclosures: **Y. Huang:** None. **K.H. Lee:** None. **J.W. Grau:** None.

Poster

066. Spinal Cord Injury: Neuroplasticity

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 66.17/N22

Topic: F.01. Human Cognition and Behavior

Support: Mildred E. Swanson Foundation

National Center for Advancing Translational Sciences of the National Institutes of Health

Michigan Institute for Clinical & Health Research Grant

Title: Intelligence and functional connectivity in people with Cerebral Palsy

Authors: *R. E. ALCAIDE[1], J. E. HUGGINS[2], S. WARSCHAUSKY[2];
[2]Physical Med. and Rehabil., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Cerebral palsy (CP) is a term that describes a spectrum of disorders of impaired motor and sensory function caused by a brain lesion occurring during early development. These lesions may also lead to intellectual disability and specific cognitive impairments. Previous studies have investigated functional connectivity in children with CP. Results have suggested that children

with CP recruit more cortical regions, have longer global network path lengths and have decreased functional connectivity in lesion areas. It is believed that, in part, these findings reflect neural compensation for underlying pathology. Increased recruitment and decreased functional connectivity are also associated with lower intelligence in typically developing individuals. This would suggest that children with CP should have lowered intelligence, however, only fifty percent of children with CP exhibit intellectual disability. Currently, it is unknown whether connectivity as reflected in brain recruitment, network path length and functional connectivity, is directly correlated with level of intellect in children with CP. Examining connectivity and cognition in children with CP is an important step in advancing our understanding of brain-behavior relations and brain reorganization following lesions to the developing brain. Using our P300 BCI-adapted Pearson Peabody Picture Vocabulary Test (PPVT-IV) on 10 typically developing children and 10 age-matched children with CP, we collected electroencephalography (EEG) data and PPVT-IV results. The PPVT-IV is a single word receptive vocabulary test that can be used as a proxy for verbal intelligence. Subjects are shown an image with four colored illustrations, and a target word is presented audibly. The subjects then select the image that best matches the audible prompt using our adapted BCI adapted PPVT-IV. The EEG data collected by the BCI were processed for network dynamics to determine recruitment, network path length and functional connectivity. PPVT-IV results were correlated between network path length, number of areas recruited and functional connectivity of each subject. Lastly, a multiple analysis of variance was used to measure differences between CP and typically developing children.

Disclosures: **R.E. Alcaide:** None. **J.E. Huggins:** None. **S. Warschausky:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.01/N23

Topic: D.12. Kinematics and EMG

Support: JSPS KAKENHI Grant Number 26-3962

Title: The effect of movement speed of visuomotor task on the activity of spinal inhibitory circuits

Authors: ***S. KUBOTA**[1,2], M. HIRANO[1,2], Y. KOIZUME[1], S. TANABE[3], K. FUNASE[1]; [1]Dept. of Human Sciences, Grad. Sch. of Integrate Arts and Sci., Hiroshima Univ., Higashi-hiroshima, Japan; [2]Res. Fellow of the Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Fac. of Rehabilitation, Sch. of Hlth. Sci., Fujita Hlth. Univ., Toyoake, Japan

Abstract: Objective: Previous studies have shown that spinal reflex circuits are modulated by motor skill training. However, the effect of task movement speed on the spinal reflex circuits have not been clarified yet. The aim of this research is to investigate whether spinal reflex circuits are affected by task movement speed. Methods: Thirty-eight healthy subjects participated in this study. In experiment 1, eighteen subjects performed visuomotor task that involve alternating ankle movement either slow (9 subjects) or fast (9 subjects) movement speed, and 9 subjects performed non-visuomotor task (control task) in fast movement speed. The movement speed was defined the time of 1 cycle of ankle movement; slow movement speed was set to 2000 ms (0.5 Hz rhythm), and fast movement speed was set to 750 ms (1.5 Hz rhythm). The amount of reciprocal Ia inhibition and presynaptic inhibition (D1 inhibition) were recorded before, immediately after, 15 min after, and 30 min after of the training session. In experiment 2, using transcranial magnetic stimulation (TMS), the effect of corticospinal descending inputs on presynaptic inhibitory pathways were examined before and after performing either visuomotor task (8 subjects) or non-visuomotor (8 subjects) task at fast movement speed condition. Results The amount of reciprocal Ia inhibition is affected by the movement speed of visuomotor task; it is increased in a fast movement speed condition, but unchanged in a slow movement speed condition. The amount of D1 inhibition is increased after visuomotor task irrespective of movement speed. Non-visuomotor task (control task) is not induced any changes in the amount of the reciprocal Ia inhibition and the D1 inhibition. TMS conditioned effect of presynaptic inhibitory pathways is changed following visuomotor task. Conclusion Corticospinal descending inputs to spinal cord during controlled limb movement are responsible for the changes in presynaptic inhibition, and that task movement speed is critical factor for inducing activity changes in reciprocal Ia inhibition in addition to corticospinal descending inputs.

Disclosures: **S. Kubota:** None. **M. Hirano:** None. **Y. Koizume:** None. **S. Tanabe:** None. **K. Funase:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.02/N24

Topic: D.12. Kinematics and EMG

Support: Neuroscience and mental health institute

Title: Reduced post-activation depression of the soleus H-reflex and root evoked potential following transcranial magnetic stimulation

Authors: *J. ANDREWS[1], R. STEIN[2], F. ROY[2];
[1]Physiol., Univ. of Alberta, Spruce Grove, AB, Canada; [2]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Post-activation depression of the Hoffmann (H)-reflex is associated with a transient period of suppression following activation of the reflex pathway. In soleus, the depression lasts for 100-200 ms during voluntary contraction and up to 10 s at rest. A reflex root evoked potential (REP), elicited following a single pulse of transcutaneous spinal stimulation to the thoracolumbar spine, has been shown to exhibit similar suppression. The present study systematically characterized the effect of transcranial magnetic stimulation (TMS) on post-activation depression using double-pulse H-reflexes and REPs. A TMS pulse reduced the period of depression to 10-15 ms for both reflexes. TMS could even produce post-activation facilitation of the H-reflex, as the second reflex response was increased to 243 ± 51% of control values at the 75 ms interval. The time-course was qualitatively similar for the REP, yet the overall increase was less. While recovery of the H-reflex was slower in the relaxed muscle, the profile exhibited a distinct bimodal shape characterized by an early peak at the 25 ms interval, reaching 72 ± 23% of control values, followed by a trough at 50 ms, and then a gradual recovery at intervals > 50 ms. The rapid recovery of two successively depressed H-reflexes, ~25 ms part, was also possible with double-pulse TMS. The effect of the TMS-induced corticospinal excitation on post-activation depression may be explained by a combination of pre- and postsynaptic mechanisms, though further investigation is required to distinguish between them.

Disclosures: J. Andrews: None. **R. Stein:** None. **F. Roy:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.03/N25

Topic: D.12. Kinematics and EMG

Title: Machine learning classification of a hemiplegic and healthy patellar tendon reflex pair through an iPod wireless gyroscope platform

Authors: *R. C. LEMOYNE[1,3], T. MASTROIANNI[2];
[1]Independent, Running Springs, CA; [2]Independent, Pittsburgh, PA; [3]Northern Arizona Univ., Flagstaff, AZ

Abstract: The attributes of the patellar tendon reflex establish fundamental insight for the conventional neurological examination. Portable media devices, such as the iPod, have been demonstrated regarding the quantification of the patellar tendon reflex response. In particular, the gyroscope sensor of the iPod provides convenient measurement of patellar tendon reflex response. A software application enables the iPod to function as a wireless gyroscope platform. A further evolution involves the incorporation of machine learning to classify a feature set of the gyroscope signal for the patellar tendon reflex response. Machine learning applications have considerable potential to advance the acuity of standard neurological examinations. A hemiplegic and healthy reflex pair is comprised of visibly disparate features regarding the patellar tendon reflex response of both legs. The iPod as a wireless gyroscope platform records and transmits the gyroscope signal of the patellar tendon reflex response as an email attachment for further post-processing through machine learning. The iPod wireless gyroscope platform with machine learning successfully classifies the disparity of a hemiplegic and healthy reflex pair.

Disclosures: R.C. LeMoyne: None. **T. Mastroianni:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.04/N26

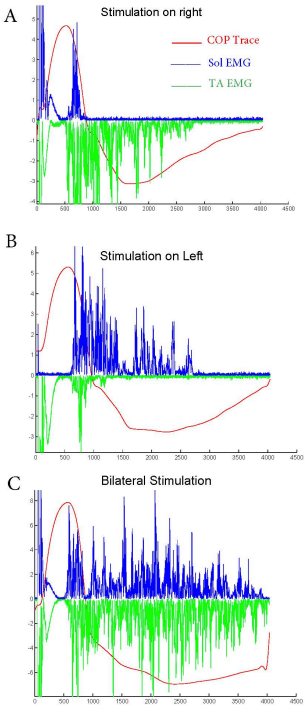
Topic: D.12. Kinematics and EMG

Title: Patterns of postural recovery in response to reflexly induced perturbation

Authors: *B. TAHAYORI[1], M. RAZEGHI[3], D. M. KOCEJA[2];
[2]Kinesiology, [1]Indiana Univ., Bloomington, IN; [3]Physical Therapy, Shiraz Univ. of Med. Sci., Shiraz, Iran, Islamic Republic of

Abstract: Control of perturbation during quiet standing entails a sequence of reactions (1). To withstand an externally applied perturbation both the muscular and the nervous system must generate proper reactions (2). In this study, we simulated an external perturbation by unexpectedly stimulating the posterior tibial nerve to cause a sudden twitch in the soleus muscle. Such a stimulation was applied to the right, left or both legs while the subjects stood on a Kistler® forceplate. Recordings from the soleus and tibialis anterior muscles were made through Ag-AgCl surface EMG electrodes. In healthy subjects, it was observed that regardless of the type of stimulation (left, right or bilateral), there was a fast forward perturbation followed by a slow recovery (showed by the red line). When stimulating the right leg, there was a brief burst of Sol with a simultaneous but long lasting contraction of TA (fig 1A). This pattern was reversed when

stimulating the left leg (and recording from the right side - fig 1B). In bilateral stimulation, both muscles showed long lasting cocontraction (fig 1C). In line with our previous studies on stroke pathology (3) we are conducting this experiment on hemiplegic patients to investigate the pattern of recovery in response to this type of experimental perturbation. References:
1.Balasubramaniam R, Wing AM. The dynamics of standing balance. Trends in cognitive sciences. 2002;6(12):531-6. 2.Cresswell A, Oddsson L, Thorstensson A. The influence of sudden perturbations on trunk muscle activity and intra-abdominal pressure while standing. Experimental Brain Research. 1994;98(2):336-41. 3.Tahayori B, Tahayori B, Koceja D. Characteristics of preceding Ia activity on post-activation depression in health and disease. Journal of neurophysiology. 2015:jn. 00132.2015.



Disclosures: **B. Tahayori:** None. **M. Razeghi:** None. **D.M. Koceja:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.05/N27

Topic: D.12. Kinematics and EMG

Title: Effects of postural orientation and weight bearing on soleus H-reflex in young and elderly subjects

Authors: *M. R. ENYART[1], A. PHIPPS[1], K. KITANO[2], D. KOCEJA[2];
[2]Kinesiology, [1]Indiana Univ., Bloomington, IN

Abstract: It is known that the soleus H-reflex is suppressed in young subjects in response to a postural orientation change from spine to upright, whereas soleus H-reflex is facilitated among the elderly during the same postural orientation change. This contrary property of the soleus H-reflex is considered to be a result of improper information processing of inputs from the vestibular system in conjunction with proprioceptive information. The purpose of the study was to investigate the effects of postural orientation change on the soleus H-reflex with change in muscle tone. Soleus H-reflexes were measured from nine participants (six young and three elderly). The two postural testing positions were supine (0°) and tilted (60°). Two conditions were investigated; weight-bearing (WB) and non-weight-bearing (NWB). In NWB, subjects were fixed on a tilt table with a harness. At 0°, H-reflexes were evoked during a static condition (subjects were stationary). At 60°, H-reflexes were tested while the tilt table was moving at 4.3°/sec. The intensity of electrical stimulation was set to evoke small M-waves approximately equal to 5% of Mmax. In order to isolate the effects of the vestibular system, visual information was occluded. Modulation ratios were calculated as percentages of control values (H-reflex amplitude at 0°). Young subjects demonstrated 92% and 320% increases (facilitation) in WB and NWB conditions, respectively, whereas elderly subjects demonstrated the opposite: 320% and 160% increases in WB and NWB. A significant interaction ($F(1,7) = 75.8$, $p<.05$) was found between age group (young vs. elderly) and weight condition (WB vs. NWB). Results demonstrated that at 60 degrees of tilt, the young subjects produced greater facilitation to the soleus motor pool in the non-weight-bearing condition, whereas the elderly group demonstrated greater facilitation when weight bearing. These results coincide with earlier studies and suggest differences in weighting of both vestibular and somatosensory inputs in young and elderly.

Disclosures: **M.R. Enyart:** None. **A. Phipps:** None. **K. Kitano:** None. **D. Koceja:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.06/N28

Topic: D.12. Kinematics and EMG

Support: FQRNT

NSERC

Title: Ipsilateral and contralateral reflex modulation during tied-belt and split-belt locomotion in spinal-transected cats

Authors: *M.-F. HURTEAU, Y. THIBAUDIER, C. DAMBREVILLE, V. KUCZYNSKI, A. FRIGON;
Physiol. and Biophysics, Univ. De Sherbrooke, Sherbrooke, QC, Canada

Abstract: During locomotion, cutaneous reflexes are modulated with task and with the phase of the step cycle to respond to perturbations. Here, we investigate the modulation of cutaneous reflexes during tied-belt (equal left-right speeds) and split-belt (different left-right speeds) locomotion in chronic spinalized adult cats. Three cats with a spinal-transection at T12-T13 level were implanted with electrodes for electromyography and to stimulate the left superficial peroneal (SP) nerve. Reflexes were evoked during tied-belt locomotion at 0.4 m/s and at 0.6 m/s and during split-belt locomotion with the slow and fast hindlimbs stepping at 0.4 m/s and 0.6 m/s, respectively. Both the left and right sides were used as the slow and fast sides. Approximately 120 single pulse stimulations were applied to the left SP nerve at 1.2 times the motor threshold throughout the cycle. The left hindlimb cycle was divided in 10 equal sub-phases. Short latency excitatory (P1) and inhibitory (N1) responses, as well as long latency excitatory responses (P2) were evaluated in ipsilateral flexors and extensors. Crossed responses were also assessed in flexors and extensors. During tied-belt locomotion, P1 and P2 responses in ipsilateral flexors (sartorius, semitendinosus, tibialis anterior) peaked during flexion. In ipsilateral extensors (vastus lateralis, lateral and medial gastrocnemius), N1 responses were observed during extension followed by P2 responses in 2 cats while in the other cat, P1 responses were observed. In all cats, P1 responses were observed in extensors during flexion. In contralateral flexors and extensors, excitatory responses were present during the flexion phase of the stimulated limb. During split-belt locomotion, patterns of reflex modulation found during tied-belt locomotion were preserved, although reflex amplitudes were reduced. Ipsilateral P1 and P2 responses in extensors were reduced by 34 % and 31.5%, respectively, during split-belt locomotion when compared to responses obtained during tied-belt locomotion at the same speed. Ipsilateral P1 and P2 responses in flexors were reduced by 22% and 17%, respectively. Contralateral responses were reduced in flexors and extensors by 25% and 32%, respectively. Therefore, during split-belt locomotion, reflex mechanisms are maintained to facilitate flexion of the stimulated limb during the swing phase while crossed reflex responses co-activate flexors and extensors, presumably to help stabilize the support limb. The reduction in reflex amplitude during split-belt locomotion might be a mechanism to decrease sensory perturbations in a less stable condition.

Disclosures: **M. Hurteau:** None. **Y. Thibaudier:** None. **C. Dambreville:** None. **V. Kuczynski:** None. **A. Frigon:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.07/N29

Topic: D.12. Kinematics and EMG

Support: Jeffress Foundation

Title: Rat hind limb nociceptive withdrawal response to heat and mechanical stimuli depends on initial paw posture but not stimulus location

Authors: ***G. VERDI**[1], T. L. BERRENA[2], K. M. SEAMON[2], C. A. CHRZAN[2], M. HARTMANN[2], B. C. GUMPERT[2], M. N. KABORE[2], K. MOORE[2], C. L. CLELAND[2]; [2]Biol., [1]James Madison Univ., Harrisonburg, VA

Abstract: Rats rapidly withdraw their hind limb in response to a noxious stimulus applied to the plantar surface of their paw, which is an example of the Nociceptive Withdrawal Response (NWR). Previous studies in spinalized or lightly anesthetized non-human mammals have shown that the spatial organization of the response depends on stimulus location. The goal of our studies was to determine if the location of heat and mechanical stimuli, or other factors such as initial posture, determines the direction of withdrawal in intact, unanesthetized rats. For testing the response to heat stimuli, rats were placed on a glass plate through which an infrared laser (980 nm) was directed to heat a small (1mm) localized portion of the plantar surface of the foot. Heat stimuli were delivered along three dimensions (rostral-caudal, lateral-medial, to each of the five toes). For testing the response to mechanical stimuli, rats were placed on a wire mesh and a stimulus (nylon monofilament or 30g needle) was applied to one of five locations along the rostral-caudal and lateral-medial dimensions on the plantar surface. The resulting withdrawal response was recorded with three conventional camcorders (60 fps), one on the left, one on the right, and the third underneath the rat. From the video beneath the rat, the initial location and angle of the stimulated paw was recorded. In response to both heat and mechanical stimuli, the rat withdrew and rapidly (~40ms) replaced its paw on the glass/mesh, at which point the final location and angle of the paw were recorded. Rats withdrew and then replaced their paw on the glass in all possible directions. To determine if the location of the stimulus influenced response direction and magnitude, the rat's paw was stimulated along each of the three dimensions. Unexpectedly, we found that the direction of response did not depend on stimulus location across

both heat and mechanical stimuli and all three dimensions of stimulus location. However, we noticed that the initial position of paw varied in both location and angle. Consequently, we explored if initial position and paw angle influenced final location and angle. Our results consistently revealed that the direction and magnitude of the response depended significantly and strongly on the initial location and angle of the foot. For example, if the paw was initially rostral, the movement tended to be caudal, and if the paw was initially medial, it would move lateral. These results demonstrate, in contrast to studies in spinalized or anesthetized non-human animals, that initial posture plays a greater role in the programming of the NWR than stimulus location.

Disclosures: **G. Verdi:** None. **T.L. Berrena:** None. **K.M. Seamon:** None. **C.A. Chrzan:** None. **M. Hartmann:** None. **B.C. Gumpert:** None. **M.N. Kabore:** None. **K. Moore:** None. **C.L. Cleland:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.08/N30

Topic: D.12. Kinematics and EMG

Support: Jeffress Foundation

Title: Dependence of the nociceptive withdrawal response of the tail on stimulus location and intensity

Authors: *Q. KANG, S. J. THAI, J. KIM, J. A. BRAUN, C. L. CLELAND;
James Madison Univ., Harrisonburg, VA

Abstract: The nociceptive withdrawal response (NWR) allows mammals to avoid harmful stimuli that could cause tissue damage. The NWR has been studied across diverse groups of animals revealing that stimulus location can influence the spatial organization of the NWR. However, previous studies in non-human animals have been conducted largely under either light anesthesia or following decerebration. The aim of this study is to determine in intact, unanesthetized rats how the nociceptive withdrawal response to localized heat stimuli depends on rostral-caudal stimulus location and stimulus intensity. Adult intact, unanaesthetized Sprague-Dawley rats were placed inside an acrylic tube with their tail protruding straight from the rear of the tube. Thirteen circular dots were marked dorsally along the length the tail with a black marker. Localized (1mm diameter) heat stimuli were delivered continuously until withdrawal

with a laser diode (980nm) to the lateral surface of the tail at the caudal 12 levels. The resulting NWR of the tail was recorded using high speed video (650 fps) positioned directly overhead. Latency was used a proxy for stimulus intensity and varied between 0.5 and 15 s. The 13 dots were tracked automatically in software to obtain the location of each of the dots in the rostral/caudal - lateral/medial plane over time. The resulting movement stayed largely within the horizontal plane. The movement of the tail was quantified in terms of tail base rotation and local bend along the length of the tail. Preliminary results revealed that rotation of the tail at its base and local bend at various levels along the tail were the primary features of the withdrawal response. The level of the local bend typical progressed caudal through the movement. The rotation of the base of tail depended non-linearly with stimulus level; magnitude increased as the stimulus moved caudal from the base but then decreased as the stimulus level approached the caudal end of tail and actually reversed in direction for stimuli delivered to the last two levels. These results suggest that movement associated with nociceptive withdrawal of the tail is both complex and depends on stimulus location.

Disclosures: Q. Kang: None. **S.J. Thai:** None. **J. Kim:** None. **J.A. Braun:** None. **C.L. Cleland:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.09/N31

Topic: D.12. Kinematics and EMG

Support: Jeffress Foundation

Title: Postural modulation of the nociceptive withdrawal response of the tail in intact, unanaesthetized rats

Authors: *J. KIM, Q. KANG, C. L. CLELAND;
Biol., James Madison Univ., Harrisonburg, VA

Abstract: The nociceptive withdrawal response (NWR) allows mammals to avoid harmful stimuli that could cause tissue damage. The NWR has been studied across diverse groups of animals revealing that both stimulus location and initial posture can influence the spatial organization of the NWR. However, previous studies in non-human animals have been conducted under either light anesthesia or following decerebration. The aim of this study is to determine in intact, unanesthetized rats how the relationship between stimulus location and

pattern of movement depends on tail curvature and rotation. Adult intact, unanaesthetized Sprague-Dawley rats were placed inside an acrylic tube with their tail protruding straight from the rear of the tube. Thirteen circular dots were marked dorsally along the length the tail with a black marker. Heat stimuli were delivered with a laser diode (980nm) to the lateral surface of the tail at three rostral-caudal levels. The posture of the tail was changed in two ways. First the tail was curved circularly with five different diameters (0, 8, 16, 32, 64 cm). Second, the tail was kept straight but the base of the tail was rotated to four different angles (0, 30, 60, 90 degrees). The resulting NWR of the tail was recorded using high speed video (650 fps). The 13 dots were tracked automatically in software to obtain the location of each of the dots in the rostral/caudal - lateral/medial plane over time. The resulting movement stayed largely within the horizontal plane. The movement of the tail was quantified in terms of tail base rotation and local bend along the length of the tail. Preliminary results from rats with variously curved tails suggest that rotation associated with the NWR around the base of the tail did not vary with tail curvature. However, the local bend was absent for the largest tail curvatures when the stimulus was applied from outside of the curve of the tail. These observations are similar to our recent results in the NWR of foot in which the response depends strongly on the initial posture of the foot.

Disclosures: J. Kim: None. **Q. Kang:** None. **C.L. Cleland:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

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Topic: D.12. Kinematics and EMG

Support: NIDR grant H133P110013-12

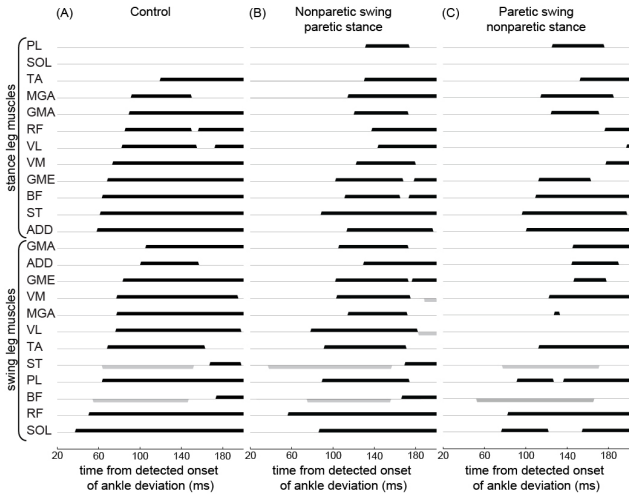
NINDS grant 5R01NS064084-02

Title: Post-stroke disruption of bilateral lower limb neuromuscular control in response to a unilateral gait perturbation

Authors: *B. SHARAFI[1], Y. Y. DHAHER[2];
[1]Ctr. for Physical Ergonomics, Liberty Mutual Inst. For Safety, Hopkinton, MA; [2]Northwestern Univ., Chicago, IL

Abstract: The goal of this study was to characterize post-stroke changes in the bilateral sequence of lower limb responses following a destabilizing unilateral perturbation during gait.

Previous studies have shown delayed reflexes in the paretic leg following balance perturbations from standing position. Investigating lower limb responses to gait perturbations may reveal post-stroke impairments of intra and inter-limb reflexes that are relevant to real-life situations in which stability is challenged. 11 stroke survivors and 8 healthy subjects walked on a treadmill. We interrupted the foot in early swing phase. Kinematics and lower limb electromyography were recorded. The interruption of the foot induced ankle dorsiflexion, followed by hip extension and knee flexion in the perturbed swing leg. In healthy subjects (Figure 1 A), the induced dorsiflexion evoked a short latency activation in the swing leg soleus (38 ms). Medium and long latency responses (50-70 ms) emerged in the swing leg rectus femoris (facilitation) and hamstrings (inhibition). Activation onsets of stance leg hamstrings and hip adductor and abductors occurred with latencies of 60-80 ms. In stroke patients, regardless of which leg was perturbed, the bilateral control sequence was disrupted (Figure 1, B and C). In the stance leg, as in healthy subjects, the earliest responses occurred in the hamstrings and the hip adductor and abductors. However, latencies were significantly ($\alpha<0.05$) longer. In both paretic and nonparetic swing legs, the short latency soleus response was absent. Disruption of the control sequence was more prominent when the paretic leg was perturbed; onsets of quadriceps responses were significantly later in the paretic swing leg than in the nonparetic swing leg. Moreover, the stance leg quadriceps activations were delayed when the paretic leg was perturbed. It is possible that the quality of the afferent input from the paretic swing leg contributes to the disruption of the bilateral control sequence. However, the altered sequence in the nonparetic perturbed leg suggests bilateral mechanisms of impairment.



Disclosures: B. Sharafi: None. **Y.Y. Dhaher:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.11/N33

Topic: D.12. Kinematics and EMG

Support: NSERC

Title: Effects of wrist orientation and level of muscle activation on the magnitude of reciprocal inhibition and cutaneous reflexes in forearm muscles

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Abstract: Amplitude modulation of reciprocal inhibition (RI) and cutaneous reflexes have been studied in various motor tasks in the human. In the lower limb, a common method to evoke RI is stimulating the antagonist mixed nerve while measuring the evoked response in the target muscle. Cutaneous reflexes have also been extensively examined to test the modulation of spinal pathways in the legs. Previous studies suggest that spinally-mediated reflex amplitudes are significantly affected by posture and muscle activation level. However, less is known about modulation of RI and cutaneous reflex amplitudes in the arm. We explored modulation of these evoked responses in extensor carpi radialis (ECR) muscle with varied contraction intensities (10, 25, 35 and 50% of maximal voluntary contraction (MVC)) and with the hand horizontal or vertical. RI was evoked by a single pulse stimulation applied to the median nerve (MED) in the upper arm. Cutaneous reflex were evoked by trains of stimulation to superficial radialis (SR) nerve or MED at the wrist. The largest effect of muscle activation level was observed for RI and was less affected by wrist position. This contrasts with results published elsewhere on forearm H-reflex amplitudes which are strongly modulated by wrist position. The relationships for cutaneous reflexes may be weaker than those observed for RI, suggesting that early latency cutaneous reflex amplitudes in ECR can be less sensitive to muscle activation level and joint orientation. We conclude that the modulation of reciprocal inhibition and early latency cutaneous reflexes may have different dependencies on the level of muscle activation and joint orientation.

Disclosures: Y. Sun: None. **E. Zehr:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.12/N34

Topic: D.12. Kinematics and EMG

Support: Heart and Stroke Foundation of Canada

Title: Topographic organization of responses evoked by discrete cutaneous stimulation of the foot dorsum during locomotion

Authors: *G. E. PEARCEY[1,2,4], T. KLARNER[1,2,4], T. S. BARSS[1,2,4], Y. SUN[1,2,4], C. KAUPP[1,2,4], T. NAKAJIMA[5], T. KOMIYAMA[6], B. MUNRO[7], E. ZEHR[1,2,4,3];
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Abstract: Stimulation of cutaneous nerve branches innervating the foot induces functional corrective responses that are phase-dependently modulated throughout the gait cycle. Cutaneous stimulation of discrete regions of the foot sole have been shown to induce specifically tuned "sensory steering" during locomotion. We hypothesized that this topographical organization would also be found on the dorsal foot surface. Non-noxious electrical stimulation was delivered to five discrete locations (base of 1st and 4th metatarsal, midfoot medial and lateral skin surfaces and ankle crease) on the dorsal surface of the foot during treadmill walking. Activity of muscles (EMG) acting at the ankle, knee, hip and shoulder joints was recorded along with kinematics at these joints. All data were sorted based on stimulus occurrence in twelve step-cycle phases, before being averaged together within a phase for subsequent analysis. The results illustrate large, dynamic and statistically significant site-specific and phase-dependent changes in reflex amplitudes and kinematics. The predominant effects of the dorsal foot stimulation were expressed during the swing phase and functionally served to maintain stability of the swinging limb. In general, responses of lateral and medial stimulation differed and the effects were most prominent at the distal end of the foot (1st and 4th metatarsals). Given the unstable nature of the swing phase, responses to stimulation go beyond the muscles that control the ankle to include muscles at the hip and shoulder. These results increase our understanding of how afferent feedback from specific cutaneous locations of the dorsum of the foot influences the mechanisms involved in swing phase corrective responses. These data may provide potential rehabilitative application to enhance function following neurological damage.

Disclosures: **G.E. Pearcey:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Industry Research Partner. **T. Klarner:** None. **T.S. Barss:** None. **Y. Sun:** None. **C. Kaupp:** None. **T. Nakajima:** None. **T. Komiyama:** None. **B. Munro:** C. Other

Research Support (receipt of drugs, supplies, equipment or other in-kind support); Industry Research Partner. **E. Zehr:** 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; Industry Research Partner. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Industry Research Partner.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.13/N35

Topic: D.12. Kinematics and EMG

Support: Heart and Stroke Foundation of Canada

Title: Can five weeks of arm cycling training improve walking and neurological integrity in chronic stroke?

Authors: *C. KAUPP[1,2,4], T. KLARNER[1,2,4], Y. SUN[1,2,4], N. ZAPOTOCZNY[1], H. CULLEN[1,2,4], T. S. BARSS[1,2,4], G. E. PEARCEY[1,2,4], E. P. ZEHR[1,2,4,3];
[2]Ctr. for Biomed. Res., [3]Div. of Med. Sci., [1]Univ. of Victoria, Victoria, BC, Canada; [4]Intl. Collaboration on Repair Discoveries, Vancouver, BC, Canada

Abstract: Strong interactions between the upper and lower body are well established across species and recent work has highlighted the implications of these connections for rehabilitation in humans. During walking, interlimb spinal networks regulate gait in healthy individuals and remain at least partially intact after stroke. What remains to be investigated is whether training the arms after stroke can facilitate these networks and result in improved walking function. The purpose of this study was to evaluate the contributions of rhythmic upper limb training to improvements in strength, reflex modulation, and walking function post stroke. Chronic stroke participants (at least 6 months post infarct) were recruited to a 5 week long (30 min @ 60rpm x 3 / wk) arm cycling intervention with a within-subject multiple baseline control. Over the course of the training, heart rate and rate of perceived exertion (RPE) were recorded in order to evaluate physiological cost. Resistance of the Sci-Fit Pro 2 ergometer was gradually increased over the five weeks in order to maintain a stable RPE. Muscle activation during treadmill walking and rhythmic arm cycling was assessed in the more affected (MA) and less affected (LA) side via surface electromyography (EMG) in the following muscles; tibialis anterior (TA), soleus (SOL), anterior deltoid (AD), biceps brachii (BB), triceps brachii (TB) and flexor carpi radialis (FCR).

Maximal voluntary isometric contraction strength was evaluated bilaterally in the legs via plantarflexion and dorsiflexion and in the arms via grip strength. In order to evaluate changes in interlimb neural coupling, cutaneous reflexes were elicited via electrical stimulation (5x1.0ms trains @ 300Hz) of the superficial radial nerve during treadmill walking. The effects of cervical networks on lumbar spinal cord excitability were assessed with bilateral soleus stretch reflexes elicited at rest and during 1Hz arm cycling. Clinical status and walking ability were determined via the six minute walk, ten meter walk, and timed up and go tests. Preliminary results show a modulation of interlimb connectivity, changes in strength and muscle activation during maximum voluntary contraction, and improvements in clinical walking function. Taken together, these results emphasize the utility for incorporation of upper limb activity in the functional rehabilitation of walking after neurotrauma.

Disclosures: **C. Kaupp:** None. **T. Klarner:** None. **Y. Sun:** None. **N. Zapotoczny:** None. **H. Cullen:** None. **T.S. Barss:** None. **G.E. Pearcey:** None. **E.P. Zehr:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.14/N36

Topic: D.12. Kinematics and EMG

Support: NSERC

Title: Effects of tonic cutaneous input on sensory feedback transmission in the upper limb

Authors: ***T. S. BARSS**[1,2,3], G. E. P. PEARCEY[1,2,3], B. MUNRO[5], E. P. ZEHR[1,2,3,4];
[1]Univ. of Victoria, Rehab Neuro Lab., Victoria, BC, Canada; [2]Intl. Collaboration on Repair Discoveries, Vancouver, BC, Canada; [3]Ctr. for Biomed. Res., [4]Div. of Med. Sci., Univ. of Victoria, Victoria, BC, Canada; [5]Nike Exploration Team Sport Res. lab, Natural Motion Div., Beaverton, OR

Abstract: Cutaneous feedback from the skin provides accurate perceptual information about joint position and movement (Edin, 1992, 2004; Collins et al., 1996, 2000, 2005). Integrated with other sensory modalities, cutaneous feedback informs judgements of position and movement around joints throughout the body. Afferent information from the skin is “tuned” by the nervous system depending upon the time of activation in the locomotor cycle and the task being completed (Zehr & Kido, 2001; Zehr, Collins, Frigon & Hoogenboom, 2003). This information goes largely unnoticed in conscious movement due to its relatively fast acting effects on motor

output. However, a constant tactile input to the skin may alter excitability through changes in pre-synaptic inhibition of muscle afferent feedback. The purpose here was to explore how tonic sustained input to the skin modulates sensory feedback transmission in the upper limb. Neurologically intact participants performed: 1) tonic muscle contractions (10% EMGmax); 2) Arm cycling @ 1Hz (60 rpm). Each of these tasks was completed under two conditions including, CONTROL (no cutaneous input), and CONSTANT (constant cutaneous input applied across the elbow joint). Outcome measures included reflex (H and cutaneous) amplitudes and background muscle activity assessed via electromyography (EMG). All data were normalized to maximum evoked M-waves. M-H recruitment curves were constructed using bipolar surface electrodes placed over the median nerve just proximal to the medial epicondyle of the humorous with single 1.0-ms pulses occurring pseudo randomly every 2 - 4 seconds. Conditioned recruitment curves were also elicited through stimulation of the superficial radial (SR) or median (MED) nerves at the wrist (3xRT for 5x1 ms @ 300Hz) 37 ms prior to median nerve stimulation above the elbow. Cutaneous reflex pathways were also evaluated through stimulation of the either the SR or MED nerve at the wrist during a 10% contraction. Reflexes during cycling were evoked at 4 specific positions in the movement cycle. EMG was recorded in the flexor and extensor carpi radialis as well as biceps and triceps brachii muscles. Results indicate that providing a constant tactile input to the skin modulates the excitability of afferent connections independent of descending input. Changes in pre or post synaptic inhibition within a limb receiving constant cutaneous input may alter the functional “set-point” of ongoing motor output.

Disclosures: **T.S. Barss:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Industry Research Partner. **G.E.P. Pearcey:** None. **B. Munro:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Industry Research Partner. **E.P. Zehr:** 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.; Industry Research Partner.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.15/N37

Topic: D.12. Kinematics and EMG

Support: National Center for Advancing Translational Sciences, National Institutes of Health 8KL2TR000056

Title: Fatiguing contractions of paretic knee extensors do not exacerbate stretch reflex responses

Authors: *R. BERRIOS, M. KIRKING, H. KUHNEN, S. HUNTER, B. SCHMIT, A. HYNGSTROM;
Marquette Univ., Milwaukee, WI

Abstract: Objective: Post stroke, hyper excitable stretch reflex responses in the leg can interfere with movement for activities such as gait. Previous studies have examined the effects of brief bouts of exercise on stretch reflex responses, but the effect of fatiguing contractions on stretch reflex responses is unknown. Clinically, this is relevant as strengthening regimens require muscle overload (fatigue). The purpose of this experiment was to investigate the effects of sub-maximal fatiguing knee extensor contractions on the magnitude of the stretch reflex response in individuals with chronic stroke. We predicted that the magnitude of the paretic patellar tendon reflex response (elicited by tendon tapping) would decrease following fatiguing contractions, but not as much as in control subjects. **Methods**: 10 subjects with chronic stroke and 10 age-matched controls participated in the study. Each subject group contained 5 females and 5 males. Subjects were seated on a System 3 Dynamometer (Biodex Medical Systems, Shirely NY) with their hips and knees at 90 degrees of flexion. A Linmot linear motor (LinMot Inc, Delavan WI) was used to tap the patellar tendon. 3-5 knee extensor maximal voluntary contractions (MVCs) were performed. Next, 5 tendon taps (2 Hz) to the patellar tendon were performed. The fatigue protocol consisted of 5 isometric knee extensor contractions (20% of MVC, 5s) and 1 MVC. This set was repeated until task failure. Immediately following the fatigue protocol, a final set of tendon taps was performed. For each individual, the peak reflex response was calculated and normalized to their baseline MVC. The percent decline between baseline and post fatigue reflex response measurements was then calculated. In addition, the post fatigue reflex response was correlated with post fatigue MVC values for both groups. **Results:** Individuals with stroke had shorter task duration (26.1 min $\pm$ 12.6) as compared to controls (40.2 min $\pm$ 14.0, p=0.03). The percent decline in peak reflex response was larger in controls (50.3% $\pm$ 34.0) as compared to the stroke group (16.3% $\pm$ 21.7, p=0.02). There was a positive correlation between the magnitude of the post fatigue reflex response and MVC for the controls (r^2=0.41), but not the stroke group (r^2= 0.003). **Conclusion**: Despite decreased task endurance, fatiguing exercises do not worsen stretch reflex responses in the paretic leg. Clinicians can utilize the fact that fatiguing exercises (necessary for overloading muscle and strengthening) will not increase hyper-reflexive activity or interfere with movement immediately following the exercise.

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Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.16/N38

Topic: D.12. Kinematics and EMG

Support: Obel Family Foundation

Spar Nord Fonden

Title: The effect of fatigue on interlimb communication

Authors: ***S. GERVASIO**, A. J. T. STEVENSON, N. MRACHACZ-KERSTING;
Aalborg Univ., Aalborg, Denmark

Abstract: Performing repeated motor tasks during a volleyball, basketball, or soccer game can lead to muscular fatigue, inducing a decrease in the capacity of a muscle to generate force, changes in motor coordination, and an alteration in motor performance. These changes have been attributed to an elevated risk of musculoskeletal injury. Little is known about if and how fatigue affects the coordination between the two legs during a long lasting game, and whether this could be one of the causes behind the increased occurrence of injuries towards the end of a game. The aim of the present study was to investigate the possible effect of muscular fatigue on interlimb communication. Eight amateur male soccer players took part to two recording sessions in which they either performed a 90-minute simulated soccer match (SAFT90) or rested (control session). Interlimb communication was investigated by quantifying short latency crossed responses (SLCR) observed in muscles of the contralateral (dominant) leg following nerve stimulation of the ipsilateral (non-dominant) leg. SLCR and maximal voluntary contraction (MVC) of the ankle plantarflexors in the dominant leg were measured before and after the SAFT90 and control session. MVC was quantified while the participants performed a plantarflexion on a force platform, with the ankle and knee joints fastened at 90^{o} . SLCR were elicited by electrical stimulation of the ipsilateral tibial nerve while the participants walked on a treadmill. The stimulation (intensity 85% Mmax) was delivered at 80% of the participant's walking cycle. SLCR in the contralateral gastrocnemius lateralis were quantified as the ratio between the root mean square (RMS) value of the averaged EMG in a time window between 65 and 95ms after the stimulation, and the RMS in the same time window of the control EMG (average of gait cycles with no stimulation). One participant did not show any decrease in MVC after the SAFT90 and was therefore excluded from further analysis. The MVC significantly decreased ($P < .05$) from 1633 ± 151 N to 1263 ± 292 N in the SAFT90 session, but not in the control session (1354 ± 391N before and 1352 ± 404 N after). No significant changes were observed in the SLCR amplitudes. However, a bigger decrease (18% decrease, from 206 ± 107 to 183 ± 85%) was observed after the SAFT90 compared to the control session (14% decrease, from 171 ± 55%

to 146 ± 34%). These preliminary results show that fatigue induces a reduction, although not significant, of the SLCR. Further testing is needed to confirm this hypothesis. Such information may be relevant for preventing musculoskeletal injuries caused by fatigue.

Disclosures: S. Gervasio: None. **A.J.T. Stevenson:** None. **N. Mrachacz-Kersting:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.17/N39

Topic: D.12. Kinematics and EMG

Title: The roles of contralateral conditioning and ipsilateral control stimulus intensities on soleus h-reflex modulation

Authors: A. M. PHIPPS[1], M. R. ENYART[2], K. KITANO[2], *D. M. KOCEJA[2];
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Abstract: H-reflex has been extensively used to investigate spinal mechanisms. When used with conditioning techniques, it can prove greater insight into specific circuitries. One should pay attention to the stimulus intensities of both conditioning and test stimulations to provide reliable assessment of modulation. Precisely, the magnitude of modulation in the soleus H-reflex induced by conditioning stimulation depends on the size of the control H-reflex as well as conditioning stimulus intensity. Whereas many studies have investigated the role of unilateral reflex conditioning the role of contralateral conditioning can uncover information about more complex neural networks. However, the influence of both the control H-reflex and the intensity of the conditioning stimulation are still unclear. Therefore, the purpose of this study was to assess effects of (1) control H-reflex size and (2) intensity of conditioning stimulation applied to the contralateral common peroneal nerve on the soleus H-reflex. Six participants were tested in the prone position. Two amplitudes of control H-reflex on the ipsilateral side and three intensities of conditioning stimulation applied on the contralateral side were tested. The conditioning stimuli, a train of four for 20ms, to the contralateral common peroneal nerve preceded the ipsilateral soleus test stimulation by 25, 50, 75, 150, and 300ms. Soleus control H-reflex was set to 50% of H-max and 15% of M-max. Three intensities were used as a conditioning stimulation (1.2, 1.0, and 0.8 X tibialis anterior motor threshold). Subjects received six conditions (2 H-reflex sizes by three conditioning intensities). At 50ms the H-reflex was modulated by -2% (inhibition), +25% (facilitation), and +51% with 0.8, 1.0, and 1.2 X MT conditioning stimuli, respectively. An ANOVA with repeated measure showed a significant difference ($F(2, 10)= 4.44$), and post-hoc

test revealed facilitation by 1.2 X MT was significantly different from that by 0.8 X MT. No significant difference was found between the sizes of the control H-reflex. These results show a stepwise increase of the conditioned soleus H-reflex amplitude as the intensity of the contralateral conditioning stimulation is increased. This suggests that small diameter afferent fibers may play a greater role in modulating the soleus H-reflex induced by contralateral common peroneal nerve stimulation.

Disclosures: **A.M. Phipps:** None. **M.R. Enyart:** None. **K. Kitano:** None. **D.M. Koceja:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.18/N40

Topic: D.16. Posture and Gait

Title: Idiopathic adolescent scoliosis; evidence of neuromuscular and respiratory alteration of motor drive as a possible etiology

Authors: ***M. M. SABBAHI**[1], M. BADGHAISH[2], F. OVAK BITTAR[3], H. ALROWAYEH[4], E. ABD EL KAFY[5], M. ALAYAT[5];
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Abstract: BACKGROUND: The etiology of Idiopathic Adolescent scoliosis (IAS) is mostly unknown. Neuromuscular alteration of the motor drive was proposed as a possible cause. H-reflex is an electrophysiological procedure to evaluate the spinal motor drive. Its amplitude is the best measure for muscular reflex excitability and nerve root function. Side-to-side H-amplitudes reflex asymmetry (RA) in IAS could indicate neuromuscular imbalance with a possible deficiency in neural drive. IAS may have respiratory dysfunctions. Reduced forced vital capacity (FVC), forced expiratory volume (FEV), and maximum voluntary ventilation (MVV) are reliable respiratory functions(RFs).PURPOSE: To measure the side-to- side H-reflex amplitude in patients with IAS to document RA and to compare these changes to the magnitude of the scoliotic curve (SC) as well as the associated RFs METHODS: Subjects (26 patients) with IAS (mean age = 15.2 ± 5.6, weight = 56.2 ± 23.5, height = 156.8 ± 11.2, Cobb angle = 15.5o ±3.9o) were examined in this study. Testing included H-reflex and RFs (FVC, FEV1, FEV1/FVC and MVV). The tibial nerve was electrically stimulated at the popliteal fossa while recording soleus

muscle H-reflex using surface electrodes. H-reflex was recorded bilaterally during lying (unloading) and standing (Loading). Recording parameters were 1-2 mV/div using 10 Hz-10Khz. filter. Five traces peak-to-peak amplitudes averaged and side-to-side (H/H) amplitude ratios were calculated. RFs were evaluated during standing while breathing normally for several cycles, followed by maximal inspiration, and a MVV. FVC, FEV1 and FEV1/FVC were evaluated. MVV was measured during a 10 seconds of fast breathing. Descriptive statistic was used to compare the results of the H-reflex and RFs. The degree of SC was measured using a scoliometer during Adam's stooping position. RESULTS: All patients showed RA with varying degrees. During lying, RA was 6-95% that increased during standing with increased SC. Smaller reflex asymmetry (6%-20%) was related with smaller amplitude on the concave side. Larger degree in RA (20-90%) showed the smaller amplitude on the convex side of SC. These results indicate alteration of the reflex activity and motor drive on bilateral vertebral column correlated with the aggravation of the SC. RFs showed mild restrictive pattern [FVC;75.09%, FEV1;70.6%, FEV1/FVC; 98.4 % of predicted values] reflecting impaired RFs. Reduced MVV (69.09% predictive values) reflects impaired respiratory muscles mechanical strength. CONCLUSIONS: Soleus H-RA in IAS indicates neuromuscular alteration of the motor drive as a possible etiology that was associated with alteration in RFs.

Disclosures: **M.M. Sabbahi:** Other; National plan for Science , Technology and Innovation (MAARIFAH) – King Abdulaziz City for Science and Technology - The Kingdom of Saudi Arabia , Award number (13- MED1319-10)". **M. Badghaish:** Other; National plan for Science , Technology and Innovation (MAARIFAH) – King Abdulaziz City for Science and Technology - The Kingdom of Saudi Arabia , Award number (13- MED1319-10)". **F. Ovak Bittar:** None. **H. Alrowayeh:** None. **E. Abd El Kafy:** Other; National plan for Science , Technology and Innovation (MAARIFAH) – King Abdulaziz City for Science and Technology - The Kingdom of Saudi Arabia , Award number (13- MED1319-10)". **M. Alayat:** Other; Umm Al Qura University, Faculty of Applied Medical Sciences, Makkah, KSA.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.19/N41

Topic: D.12. Kinematics and EMG

Support: 5R24HD0500821-09

Title: Double-step torques reveal a fixed 50 ms delay in integrating multi-joint motion for corrective action

Authors: *I. KURTZER;
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Abstract: Sudden limb displacement evokes a complex pattern of compensatory muscle activity. The earliest burst is generated by spinal circuits (short-latency reflex = 20-45 ms) whereas subsequent bursts are generated by spinal and cortical circuits (long-latency reflex = 50-100 ms; voluntary reaction > 120 ms). Cortical processing allows muscles to respond to the motion of the whole arm rather than local stretch information. However, it is unknown whether cortical circuits play an integrative role throughout an evolving corrective action, or whether the integrative role is delegated from cortical to spinal circuits (i.e. propriospinal neurons). If cortical circuits retain the same role, then a double step torque will evoke an integrative response to each perturbation at the same ≈50 ms delay. Alternatively, an integrative response of ≈20 ms delay to the second perturbation would point to a spinal contribution. Twelve healthy subjects (mean age = 25.5 yro; 7M & 5F) participated in an experiment testing between these possibilities. Surface EMG was obtained from their shoulder extensor (posterior deltoid) while a programmable robot (KINARM, BKiN Technologies) applied mechanical loads in two different directions: a shoulder flexion torque to flex the shoulder and a shoulder-elbow flexion torque to flex the elbow. Perturbations were applied either as a step torque or double-step torque in the same direction (35 ms, 60 ms, and 110 ms offset). Note that the step torque and double-step torques had the same initial magnitude and were randomly intermingled so we could examine the evoked response to the unexpected second perturbation. The first perturbation in the series evoked a short-latency reflex based on local stretch (i.e. when shoulder displacement evoked shoulder muscle activity, $p < 0.001$) and a long-latency reflex that integrated multi-joint motion (i.e. when elbow displacement evoked shoulder muscle activity, $p < 0.001$). All double-steps of shoulder displacement evoked a short-latency response in the shoulder muscle aligned to the second perturbation onset ($p < 0.05$). Critically, all double-steps of elbow displacement only evoked a long-latency response in the shoulder muscle aligned to the second perturbation onset ($p < 0.001$). A running receiver-operating-curve found that shoulder muscle responses had a ≈25 ms delay to the second shoulder displacements and a ≈55 ms delay to the second elbow displacements. Accordingly, the nervous system utilizes cortical circuits to integrate multi-joint motion throughout a corrective action.

Disclosures: I. Kurtzer: None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.20/N42

Topic: D.12. Kinematics and EMG

Title: Motor equivalence during whole body reaching in healthy young adults

Authors: *Y. TOMITA[1,2], A. G. FELDMAN[3,2], M. F. LEVIN[1,2];
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Abstract: The large number of kinematic degrees of freedom (DFs) of the body allows tasks to be accomplished using different combinations of DFs (motor equivalence). Despite this redundancy, certain movement features remain invariant. For example, hand trajectory and endpoint precision remained invariant when trunk movement was unexpectedly blocked during reaching from a sitting position. We hypothesized that hand trajectory and endpoint precision may remain invariant regardless of unexpected postural perturbations during whole body reaching from standing, which involves a larger number of DFs. Five healthy young subjects moved the hand to a remembered target located beyond the arm length with their eyes closed during standing (Free-Hip trials; FH). In randomly chosen trials, hip flexion was unexpectedly prevented by an electromagnetic device, forcing the subject to take a step while reaching (Blocked-Hip trials; BH). Reaching movements were also recorded when subjects intentionally made a step (Intentional-Step trials; IS). Upper/lower limb and trunk kinematics and ground reaction forces were recorded. Endpoint trajectory, joint kinematics and shifts in the postural center of pressure were analysed. Reaching trajectories and endpoint precision were invariant between FH and BH trials, while those of IS trials in some subjects differed. Invariance in the endpoint trajectory between FH and BH trials was maintained by changes in elbow/shoulder interjoint coordination patterns which occurred after the time of endpoint peak velocity. Stepping reactions resulting from the perturbation were also initiated after the time of the endpoint peak velocity in BH trials. Thus, unexpected recruitment of additional DFs that challenges postural stability during reaching from a standing position did not affect hand trajectories and endpoint precision. Movement adaptation occurred after the endpoint peak velocity to maintain invariance in endpoint trajectories, confirming our hypothesis that the nervous system can take advantage of the redundancy in the number of DFs to stabilize task performance, resulting in motor equivalence.

Disclosures: Y. Tomita: None. **A.G. Feldman:** None. **M.F. Levin:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.21/N43

Topic: D.12. Kinematics and EMG

Title: Control of posture and movement with respect to gravity by setting the referent orientation of the body

Authors: *A. MULLICK[1,3], S.-C. HSU[3,2], S. K. SUBRAMANIAN[3,4], N. TURPIN[3,4], A. G. FELDMAN[3,4], M. F. LEVIN[1,3];
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Abstract: It has been suggested that multiple muscles of the body are controlled as a coherent unit based on the difference between the referent body configuration specified by the brain and the actual, body configuration. The referent configuration is the configuration at which muscles begin to be activated. We suggest that there are referent body configurations that are used to orient actual body postures (straight or leaned) during standing and most body movements in humans and animals relative to gravity. In other words, we suggest that neural control levels can specify a referent body orientation (R) at which multiple muscles of the body reach their activity minimum. Under the influence of gravity, the body is deflected from the referent direction to a direction Q, at which the emerging muscle activity results in forces that balance gravitational forces and body stability is maintained. This hypothesis predicts that during back-and-forth movements, R and Q may momentarily coincide, bringing the activity of multiple muscles of the body to a minimum. We tested this prediction by analyzing the activity of multiple body muscles during rhythmical changes in the body orientation during standing. To manipulate R and create situations in which minima occur, participants were required to stand on 3 surface orientations, ascending, horizontal, and descending, and perform whole body sways and mini squats. EMG minima were identified from EMG signals recorded in 11 muscles across the body and the body postures at which EMG minima occurred were determined. Global minima were identified in all conditions, both in swaying and squatting movements. The EMG minima occurred in 20%-82% of movement cycles. The postures at which global EMG minima occurred were different in different conditions. Preliminary results thus confirm the hypothesis. This study contributes to the understanding of how posture and movement are controlled in the gravitational field. It also extends the previous suggestion that multiple muscles of the body are controlled as a coherent unit depending on the difference between the actual and referent configurations of the body.

Disclosures: A. Mullick: None. **S. Hsu:** None. **S.K. Subramanian:** None. **N. Turpin:** None. **A.G. Feldman:** None. **M.F. Levin:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.22/N44

Topic: D.12. Kinematics and EMG

Support: NSERC

CIHR

Heart and Stroke Foundation of Canada

REPAR MENTOR

Title: Threshold position resetting suppresses both stretch reflexes and background muscle activity in arm muscles in response to prolonged muscle lengthening

Authors: N. A. TURPIN[1,3], R. RAHAL[2,3], S. K. SUBRAMANIAN[1,3], M. F. LEVIN[4,3], *A. G. FELDMAN[1,3];
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Abstract: Since the work of Sherrington (1906), it has been recognized that, together with other proprioceptive reflexes, the stretch reflex (SR), i.e. position- and velocity-dependent resistance to muscle lengthening, plays a fundamental role in the control and stability of posture and movement. The spatial threshold of the SR, i.e. the muscle length or respective joint angle at which the SR begins to act, is broadly regulated by spinal and supra-spinal systems in a task-specific way. We tested the hypothesis that the SR threshold can be reset to suppress both SR reactions and background muscle activity in response to high amplitude lengthening, thus preventing overstretching active sarcomeres. The forearm and hand of subjects (n=12) were placed on a horizontal manipulandum. Elbow flexor or extensor muscles were pre-activated by compensating an external load (1-3 Nm) applied to the manipulandum by a torque motor. The muscles were stretched by rotating the manipulandum by 60° at different velocities (8-120°/s) randomly selected for each trial. EMG signals (biceps brachii, brachioradialis, triceps brachii lateralis and medialis), displacement and velocity were recorded. In training trials and subsequent experimental trials, subjects were instructed to abstain from intentionally modifying their responses to perturbations. Trials in which subjects changed EMG levels prior to stretch onset were excluded. SR responses to muscle stretch (lengthening), if present, were minimal and

occurred at latencies of 25-35 ms and after about 70 ms the EMG activity was suppressed for about 80 ms. After that, the stretched muscles were reactivated during the ongoing muscle lengthening. Results are consistent with the notion of resetting of spatial thresholds for muscle activation, rather than with suppression (gating) of the SR in time. In general, threshold resetting is used not only to prevent muscle overstretching but also in the control of intentional movement. By resetting thresholds, the nervous system converts posture-stabilizing to movement-producing mechanisms, thus solving the classical posture-movement problem. The possible relationship between the notion of SR threshold resetting and the clasp-knife phenomenon in some neurological conditions is discussed.

Disclosures: **N.A. Turpin:** None. **R. Rahal:** None. **S.K. Subramanian:** None. **M.F. Levin:** None. **A.G. Feldman:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.23/N45

Topic: D.12. Kinematics and EMG

Support: NSERC

CIHR

Title: The long-latency stretch response accounts for kinematic redundancy of the arm

Authors: ***J. WEILER**, P. GRIBBLE, A. PRUSZYNSKI;
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Abstract: Many studies have shown that muscle activity in the long-latency epoch (i.e., 50-100 ms after mechanical perturbation onset) displays goal-dependent modulation that shares many of the sophisticated characteristics of voluntary motor control. These observations are inline with the idea that voluntary motor actions are accomplished by the intelligent manipulation of sensory feedback. One notable capacity of voluntary motor control is its ability to take advantage of kinematic redundancy. For example, patterns of joint variance during pistol shooting appear structured to keep the shot on target. We hypothesize that the intelligent manipulation of sensory feedback also accounts for kinematic redundancy that is afforded to voluntary motor actions. We tested this hypothesis by randomly applying a flexion or extension step-torque to participants' (n=17) elbow or wrist (i.e., perturbation), which displaced the hand either into or away from a

target. Participants were required to quickly move their hand into the target following perturbation onset. Notably, the positioning of the targets were such that a perturbation applied to the elbow that displaced the hand away from the target could be counteracted by a volitional movement of the wrist, and vice versa. We found that participants responded to applied elbow or wrist perturbations that moved the hand away from the target with concurrent elbow and wrist movement. In addition, muscles that were stretched because they articulated the mechanically perturbed joint showed increased activity within the long-latency epoch when the hand was displaced away from the target compared to into the target. Critically, musculature that did not articulate the perturbed joint also displayed increased activity within the long-latency epoch when that muscle acted to move the joint in a manner that assisted transporting the hand into the target. This latter finding demonstrates that kinematic redundancy is incorporated into the intelligent manipulation of sensory feedback used to execute goal-directed actions. We are now testing whether muscle activity within the long-latency epoch accounts for kinematic redundancy when the wrist, elbow and shoulder are concurrently perturbed for reaching movements in the horizontal plane.

Disclosures: J. Weiler: None. **P. Gribble:** None. **A. Pruszynski:** None.

Poster

067. Reflexes and Reflex Modulation

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Topic: D.12. Kinematics and EMG

Support: NIH Grant NS053813-08

NSF Grant 0932263

Title: Heightened attention to proprioceptive feedback is not sufficient for long-latency reflex modulation during arm posture

Authors: *E. H. E. WALKER[1,4], R. RUIZ-TORRES[2], E. J. PERREAULT[1,4,3], L. E. MILLER[1,3,2];
[1]Biomed. Engin., Northwestern Univ., Evanston, IL; [2]Physiol., [3]Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [4]Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL

Abstract: When the arm maintains a posture against destabilizing forces, such as when holding a dumbbell overhead against gravity, the relevant muscles demonstrate an increased sensitivity of long-latency reflexes (Krutky et al. 2010). Although the involvement of motor cortex is known to be crucial for this reflex modulation (Shemmell et al. 2009), the factors that drive cortical regulation of the reflex remain unknown. Instabilities likely induce heightened arousal or attention, and studies suggest that attention can increase ascending sensory drive (Staines et al. 2000) and that mental arousal may enhance stretch reflexes (Rossi-Durand 2002; McIntyre et al. 2004). However, it is unclear whether interaction with the unstable environment is required, or if heightened attention alone is sufficient to modulate reflexes. Our main objective was to test the hypothesis that reflex sensitivity is increased due to heightened attention alone; particularly attention to proprioceptive feedback. We tested reflexes during maintenance of elbow posture while subjects attended to proprioceptive or visual stimuli; a control condition with no attention requirement was also tested. A two-alternative force choice (2AFC) task with adaptive stimulus intensities ensured continuing attention. Meanwhile, occasional ramp-and-hold perturbations were used to elicit stretch reflexes. In a subset of subjects, H-reflexes and somatosensory evoked potentials were also assessed as further probes of reflex pathway sensitivity. Our results showed that attention alone is not sufficient for reflex modulation. Although subjects demonstrated appropriate attention (proprioceptive, visual, or none) through their 2AFC responses, there were no detectable differences in reflex sensitivity for either short-latency ($p \geq 0.11$), early long-latency ($p \geq 0.64$), or late long-latency ($p \geq 0.20$) reflex periods in any monitored muscles. H-reflexes and SEPs also remained statistically equivalent in all attention conditions ($p=0.32$; $p=0.85$). This suggests that reflex modulation during a postural arm task requires more than simply heightened attention. Our paradigm effectively decoupled the attention and the postural task, such that attention was not required for purposes of stabilization, but merely for a discriminatory response. We conclude that engagement in the motor task is crucial for the type of reflex modulation that has been observed during destabilized arm posture.

Disclosures: E.H.E. Walker: None. **R. Ruiz-Torres:** None. **E.J. Perreault:** None. **L.E. Miller:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.25/N47

Topic: D.12. Kinematics and EMG

Title: Muscle innervation patterns for human wrist control as biofeedback signals for robotic rehabilitation

Authors: *A. CUPPONE[1], M. SEMPRINI[1], V. SQUERI[1], J. KONCZAK[2];
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Abstract: Robotic rehabilitation techniques typically provide extrinsic feedback to a human patient with the goal to improve motor outcomes and to aid recovery. However, feedback can be bi-directional, that is, the rehabilitation device may receive feedback about the motor performance or related physiological signals of the human user. We here focus on the usefulness of normal and abnormal muscle activation patterns as potential sources of biofeedback for controlling and optimizing force output of a robot. Before using user-generated electromyographic signals (EMG) as biofeedback, it is imperative to consider that a) the muscular redundancy found in many human limb systems and b) force generation of human muscles is not linear. Thus, it becomes important to understand how synergistic muscular control influences joint force and joint kinematics. It is the purpose of this study to characterize the electromyographic activation patterns of the major human muscles involved in controlling the hand/wrist system. By means of a robotic exoskeleton, the electromyographic activity underlying the three degrees of freedom (DoF) of hand motion was separately evaluated: wrist flexion-extension, wrist adduction-abduction and forearm pronation-supination. For each DoF four different task conditions were employed: free movements, passive movements with open eyes, passive movements with closed eyes and resistive movements. We recorded surface EMG activity of 7 muscles of the arm and forearm of healthy adult subjects. We created a lookup table by linking kinematics parameters (such as movement duration, linearity and peak velocity) with the root mean square value of the EMG signals calculated for different episodes during movement execution. This lookup table allows for identifying prototypical wrist movement patterns across the three DoF and will subsequently be integrated with the software controlling the robotic exoskeleton during experiments with motor impaired patients in order to provide them with haptic force feedback delivered when needed and with the proper amount of assistance.

Disclosures: **A. Cuppone:** None. **M. Semprini:** None. **V. Squeri:** None. **J. Konczak:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 67.26/N48

Topic: D.12. Kinematics and EMG

Support: ESPRC

MRC

Title: A geometric model of defensive peripersonal space

Authors: ***R. J. BUFACCHI**[1], M. LIANG[3], L. D. GRIFFIN[2], G. D. IANNETTI[1];
[1]Neuroscience, Physiol. and Pharmacol., [2]Computer Sci., UCL, London, United Kingdom; [3]Sch. of Med. Imaging, Tianjin Med. Univ., Tianjin, China

Abstract: The defensive peripersonal space (DPPS) is a protective area surrounding the body. Within this space, potentially harmful stimuli elicit stronger defensive reactions. However, the spatial features of the DPPS are poorly defined, and limited to descriptive estimates of its extent along a single dimension. Here we postulated a family of geometric models of the DPPS, to address two important issues related to its spatial features: What is its fine-grained topography? How does the nervous system represent the body area to be defended? These models are based on a set of assumptions about how the nervous system represents threatening stimuli, and their formalization supports the concept that when a threatening stimulus is closer to an individual, its potential for harm is increased, resulting in a stronger defensive reaction. As a measure of the DPPS, we used the strength of the defensive blink reflex elicited by electrical stimulation of the hand (hand-blink reflex, HBR), which is reliably modulated by the position of the stimulated hand in egocentric coordinates. The best fitting model indicated that (1) the nervous system's representation of the body area defended by the HBR can be approximated by a half-ellipsoid centred on the face, and (2) the DPPS extending from this area has the shape of a bubble elongated along the vertical axis. This modelling approach can be generalised to describe the spatial modulation of any defensive response.

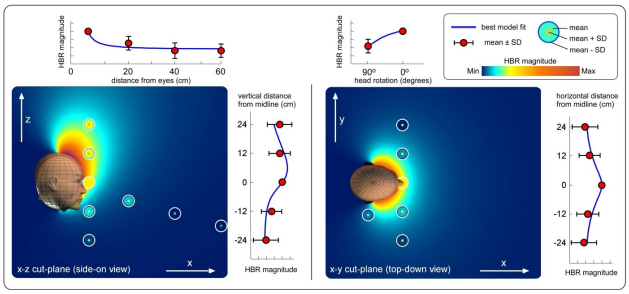

Figure 1. Effect of hand position on HBR magnitude, and geometrical modelling of DPPS. Combined description of the experimental data with the best fitting geometrical model. The measured HBR data (mean±SD) are represented as concentric circles located where the measurements were taken. The background colour represents the HBR magnitude predicted by the best-fitting geometric model. The line graphs at the side of each colour plot show HBR

magnitudes (mean±SD) along each axis, together with the best fitting geometric model (blue line).

Disclosures: **R.J. Bufacchi:** None. **M. Liang:** None. **L.D. Griffin:** None. **G.D. Iannetti:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

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Topic: D.12. Kinematics and EMG

Support: NIH Grant R01 NS053813

NIH Grant R00 HD073240

Title: Influence of task complexity on brainstem contributions to motor execution after stroke

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Abstract: The startReact response, an involuntary release of a planned ballistic motor action triggered by a startling acoustic stimulus, has been used as a non-invasive means to probe brainstem function and its contributions to motor planning and execution. We recently demonstrated that stroke survivors have intact startReact responses, paving the way to study their capacity to plan and execute ballistic movements [1]. Tasks studied to date, however, have been quite simple, involving single degree of movements in gravity-compensated environments. Stroke survivors have difficulty compensating for gravity and coordinating the actions of multiple joints. These more demanding tasks involve a spread of motor cortical activity well beyond that observed in unimpaired subjects [2]. Given the important role of the motor cortical areas in regulating brainstem activity, it is unclear if the startReact response would be present in stroke survivors performing the more demanding tasks that are integral to many activities of daily living. Our objective was to quantify how task complexity alters the startReact response following stroke. Our hypothesis was that challenging tasks that engage large areas of the motor cortex would alter the ability to activate brainstem pathways for rapid motor execution. We

exploited the well-documented abnormal motor synergies coupling shoulder antigravity muscles and elbow flexor muscles following stroke. Data were collected from 11 stroke survivors and 8 age-matched controls. Ballistic elbow extension movements were performed with and without a requirement of activating the shoulder abductors. The task involving abduction was challenging for stroke subjects who typically couple shoulder abduction with elbow flexion. Our primary finding was that the probability of eliciting a startReact response was lower for stroke subjects performing the reaching task during volitional shoulder abduction (0.73 (0.23): Mean (SD)) than when performing the same task without the need for simultaneous shoulder activity (0.43 (0.33)). The difference between tasks was significant for the stroke subjects ($\Delta = 0.30$; p=0.0003). In contrast, no significant difference was observed for the control group ($\Delta = 0.01$; p=0.53). These results indicate that cortical lesions due to stroke can significantly influence the ability to regulate brainstem pathways in a task dependent manner. [1] Honeycutt CF, Perreault EJ (2012) Plos One 7. [2] Yao J, Chen A, Carmona C, Dewald JPA (2009) Neuroimage 45:490-499.

Disclosures: H. Lee: None. **C. Honeycutt:** None. **R.L. Heckman:** None. **E.J. Perreault:** None.

Poster

067. Reflexes and Reflex Modulation

Location: Hall A

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Topic: D.12. Kinematics and EMG

Support: Ministry of Education, Culture, Sports, Scienceand Technology of Japan (#24390431 to M.I.), by the StrategicYoung Researcher Overseas Visits Program for Accelerating BrainCirculation (S2504) from the Japan Society for the Promotion of Science

Title: Effect of sodium channel blockers on the initiation of swallows in anesthetized guinea pigs

Authors: *K. TSUJI[1], T. TSUJIMURA[1], M. INOUE[1], B. J. CANNING[2];
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Abstract: Purpose: Swallowing is probably initiated by the activation of the afferent nerves that are similar to those that regulate coughing. Local anesthetics have been reported to have antitussive effects and this is due to blocking the sodium channels in sensory nerves. However, the effects of these anesthetics on initiation of swallow remains unclear. The aim of this study is to clarify the effect of sodium channel blockers on initiation of swallowing. Method(s): Ninety-

three urethane-anesthetized male guinea pigs (1.5 g/kg, i.p.) were used. Swallows were evoked by esophageal water perfusion (280 μl/min) and confirmed by electromyographic bursts from the suprahyoid muscles and increases in esophageal pressure. Drugs were added to the perfusate water. The number of swallows was measured during 30 minute water perfusion among the conditions. Effects of intraluminal drug administration on esophageal muscle contraction evoked by vagus nerve stimulation were then evaluated. Finally, changes of afferent nerve activities during esophageal distention with or w/o intraluminal drugs administration were examined *in vitro*. Result(s): Swallows were constantly evoked by esophageal water perfusion. The number of swallows w/o drugs administration was 116.6 ± 15.1. Sodium channel blockers inhibited swallowing initiation evoked by esophageal distention, in that the number of swallows was 66.1 ± 16.5 (1 mM Lidocaine), 48.3 ± 9.5 (0.1 mM Mexiletine), and 47.6 ± 11.2 (1 mM Ambroxol). Intraluminal sodium channel blockers did not inhibit esophageal muscle contraction and afferent nerve activities. Conclusion: Sodium channel blockers may inhibit swallows via not mechanosensitive afferent fibers in the myenteric plexus of esophagus, but mucosal afferent sensory nerves.

Disclosures: **K. Tsuji:** None. **T. Tsujimura:** None. **M. Inoue:** None. **B.J. Canning:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

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Program#/Poster#: 68.01/O3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: ALSA

MDA

Title: ER-mitochondria communication breakdown in ALS

Authors: ***H. KAWAMATA**[1], C. KONRAD[2], A. ARREGUIN[2], G. MANFREDI[2];
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Abstract: The endoplasmic reticulum (ER) and mitochondria are major regulators of calcium homeostasis. Numerous lines of evidence suggest that dysfunction of both mitochondria and ER participates in the pathogenesis of neurodegenerative conditions, including amyotrophic lateral sclerosis (ALS). Since these organelles are physically and functionally intertwined, it is logical

to investigate ER-mitochondria interactions to understand their roles in ALS pathogenesis. In many cell types, including astrocytes, the ER is a reservoir for intracellular calcium. Calcium released from the ER regulates a multiplicity of calcium-dependent systems. Mitochondria take up calcium, in a membrane potential dependent manner. Calcium entry in mitochondria boosts oxidative phosphorylation, as the dehydrogenases of the Krebs cycle are stimulated. Overall, the rebalancing of cytosolic calcium after ER release is an energy consuming process, since the calcium pumps that extrude calcium or refill the ER require ATP and mitochondria expend membrane potential to take up calcium. We had shown that astrocytes from SOD1 mutant mice have exaggerated ER calcium stores, which cause abnormal intracellular calcium signaling (Kawamata et al. J. Neurosci., 2014). We had also shown that skin fibroblasts from ALS patients have increased mitochondrial membrane potential and mitochondrial mass (Kirk et al. Ann. Neurol., 2014). Here, we investigated the mechanistic relationship between ER calcium handling and mitochondrial function. In mutant SOD1 astrocytes, we found an increase of mitochondrial membrane potential, similar to that found in fibroblasts from sporadic and familial ALS patients. In fibroblasts from sporadic and different forms of familial ALS, we found ER calcium dynamics abnormalities similar to those found in mutant SOD1 astrocytes. Importantly, there was a significant correlation between calcium alterations and bioenergetics, including membrane potential, oxygen consumption and glycolysis, suggesting that ER calcium alterations and energy metabolism are mechanistically related. Surprisingly, in ALS cells, ATP content was not increased, despite the higher energy metabolism, suggesting greater ATP consumption. Based on these findings, we propose a common pathway of dysregulation in sporadic and familial ALS, which involves abnormal ER calcium handling that triggers increased energy metabolism, but also higher energy expenditure. This mechanism could cause the bioenergetic machinery to work at its higher functional limits, thereby lowering the threshold for life threatening bioenergetic crises in high-energy demanding cells, such as neurons and astrocytes.

Disclosures: **H. Kawamata:** None. **C. Konrad:** None. **A. Arreguin:** None. **G. Manfredi:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.02/O4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Supported by the intramural program of NIH, National Institute of Neurological Disorders and Stroke

Title: Motor Physiology in C9orf72 amyotrophic lateral sclerosis-frontotemporal dementia

Authors: *M. FLOETER[1], T. J. LEHKY[2], L. BRAUN[2], D. BAGEAC[2], B. TRAYNOR[3], O. SCHANZ[2];
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Abstract: BACKGROUND: Persons with a repeat expansion mutation in the gene C9ORF72 may have clinical features of frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), or a combination of ALS and FTD. Non-invasive physiological measures of upper (corticospinal) and lower motor neuron function have been used to monitor disease progression in clinical trials and in animal models of ALS. It is uncertain whether such motor physiology measures reflect disease progression across the spectrum of C9orf72 clinical phenotypes. OBJECTIVE: To determine whether non-invasive physiological measures of upper or lower motor neuron dysfunction correlate with clinical findings in persons with a repeat expansion in the C9orf72 gene. DESIGN/METHODS: Clinical and physiological measures were made from 21 patients and asymptomatic carriers with a confirmed mutation at baseline, with follow-up visits at 6- and 18-months in a subset. Clinical signs and symptoms of motor and cognitive testing were carried out at each visit. Clinical measures included the Dementia rating scale, ALS functional rating scale, finger and foot tapping rate, 9-hole peg test times, and timed gait. Physiological measures included transcranial magnetic stimulation (TMS) measures of cortical thresholds, silent periods, and central motor conduction times; electrical impedance myography (EIM) of 8 muscles of the arm and leg on one side; and the compound muscle action potential (CMAP) and motor unit index (MUNIX) from the thenar muscles bilaterally. RESULTS: Compared to healthy controls, the cortical threshold was reduced in the C9+ group, particularly in patients with an ALS phenotype. Lower cortical thresholds were associated with shorter cortical silent periods and relatively preserved finger tapping speed. In patients returning at 6-months, cortical thresholds were stable. EIM, MUNIX, and CMAP declined, consistent with findings of amyotrophy. CONCLUSION: These findings point to motor cortical hyperexcitability in C9orf72 patients. Because it is more prominent in those patients with signs of ALS who still have good dexterity, it is likely to be an early event in the disease process.

Disclosures: M. Floeter: None. **T.J. Lehky:** None. **L. Braun:** None. **D. Bageac:** None. **B. Traynor:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent applied for C9orf72 gene. **O. Schanz:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.03/O5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant 5T32AG000255

NIH Grant R37NS060698

Title: Elevated CDK5 activity leads to dysregulated axonal transport in neurons

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Abstract: Axonal transport is essential for neuron function and survival, and defects in transport are associated with neurodegenerative disease including Amyotrophic Lateral Sclerosis (ALS). Increased activity of cyclin dependent kinase 5 (CDK5) and its stress-induced activator p25 is observed in human patients with ALS and in mouse models of the disease. CDK5 modulates both anterograde (kinesin) and retrograde (dynein) motor proteins, but the mechanisms of regulation remain unknown. Here, we show that expression of p25 significantly increases the number of nonprocessive events across a wide range of organelles including autophagosomes, lysosomes, mitochondria, and TrkB signaling endosomes. Similar defects are observed in $SOD1^{G93A}$ neuron, wherein reducing CDK5 activity partially rescues transport. We demonstrate that the effects of aberrant CDK5 activation depend on the Lis1/Ndel1 complex, which directly regulates dynein activity. Tight binding of Ndel1 to Lis1 requires CDK5 phosphorylation of Ndel1. Mutating the phosphorylation sites in Ndel1 prevented CDK5-mediated transport disruption. Moreover, *in vitro* imaging reveals that activated CDK5 increases dynein binding to microtubules. Together, these studies identify CDK5 as a Lis1/Ndel1-dependent regulator of cargo transport in stressed cells, and suggest that dysregulated CDK5 contributes to transport disruption in disease.

Disclosures: E. Klinman: None. **E.L.F. Holzbaur:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.04/O6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MEXT KAKENHI Grant Number 26111730

Title: A novel link between C9ORF72 expansion and phosphorylated TDP-43 aggregation

Authors: *T. NONAKA, G. SUZUKI, F. KAMETANI, M. HASEGAWA;
Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan

Abstract: An expanded hexanucleotide repeat in C9ORF72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Unconventional non-ATG translation of the expanded hexanucleotide repeat produces dipeptide repeat proteins (DPRs), which form insoluble, ubiquitinated, p62-positive aggregates that are most abundant in the cerebral cortex and cerebellum. Besides accumulation of DPR proteins, the second pathological hallmark in brains of patients with C9ORF72 mutation is the accumulation of phosphorylated TDP-43. Although the presence of DPR and TDP-43 co-accumulation in the same neurons was found to be a rare event, recent studies suggest that DPR pathology precedes TDP-43 accumulation and might trigger TDP-43 aggregate formation in a subset of neurons. To explore the link between C9ORF72 expansion and TDP-43 aggregation, we investigated whether DPR aggregation induces intracellular TDP-43 alterations using cultured cells. Synthetic cDNAs encoding 100 repeat of DPR (GFP-tagged poly-GA, -GP, -GR, -PA or -PR) without a hexanucleotide repeat sequence were prepared and transfected into SH-SY5Y cells to examine the effects of these proteins on these cells. We found that poly-GA protein was most aggregation-prone of these DPRs, and that poly-GA protein aggregates were immunoreactive with ubiquitin and p62. Surprisingly, these inclusions were co-localized with cytoplasmic aggregates composed of endogenous phosphorylated TDP-43. We also observed a few phosphorylated TDP-43 aggregates in cells expressing poly-PA or -PR. Immunoblot analyses of sarkosyl-insoluble fraction prepared from cells expressing poly-GA revealed that endogenous TDP-43 was aggregated with abnormal post-translational modifications such as phosphorylation at Ser409/410 and fragmentation. On the other hand, tau or alpha-synuclein was not accumulated in the presence of poly-GA inclusions, suggesting that poly-GA specifically induces TDP-43 aggregation. These results suggest that DPR aggregation leads to TDP-43 alterations, providing new insights into the relationship between C9ORF72 mutation and aberrant TDP-43 aggregation.

Disclosures: **T. Nonaka:** None. **G. Suzuki:** None. **F. Kametani:** None. **M. Hasegawa:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.05/O7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Girotondo ONLUS

Smarathon ONLUS

University of Turin

Title: Involvement of muscle-specific mirna-206 in the spinal muscular atrophy pathogenesis

Authors: *M. M. BOIDO[1], V. VALSECCHI[1], E. DE AMICIS[1], A. PIRAS[2], A. VERCELLI[1]; [1]Univ. of Turin, Orbassano (TO), Italy; [2]Karolinska Institutet, Stockholm, Sweden

Abstract: Spinal muscular atrophy (SMA) is a fatal paediatric genetic disease, characterized by motor neuron (MN) death, leading to progressive muscle weakness, respiratory failure, and, in the most severe cases, to death. SMA is due to the deletion or mutation of the telomeric survival MN gene (SMN1), on chromosome 5. Its homologous, SMN2 gene, only produces a limited amount of functional protein which can modulate SMA severity. Specific or general changes in the activity of ribonucleoprotein containing micro RNAs (miRNAs) play a role in the development of SMA. Additionally miRNA-206 has been shown to slow amyotrophic lateral sclerosis progression by promoting a compensatory regeneration of neuromuscular synapses. Therefore, we correlated the morphology and the architecture of the neuromuscular junctions (NMJs) of the quadriceps, a muscle which is affected early in SMA, with the expression levels of miRNA-206 in a murine model of intermediate SMA (SMA II). Our results showed a decrease in the percentage of type II fibers, an increase in atrophic muscle fibers and a remarkable accumulation of neurofilament (NF) in the pre-synaptic terminal of the NMJs in the quadriceps of SMA II mice. Furthermore, molecular analysis highlighted a direct link between miRNA-206 - HDAC4 - FGFBP1, and in particular, a strong up-regulation of this pathway in the late phase of the disease. We propose that miRNA-206 is activated as survival endogenous mechanism, although not sufficient to rescue the integrity of motor neurons. We speculate that early modulation of miRNA-206 expression might delay SMA neurodegenerative pathway and that miRNA-206 could be an innovative, still relatively unexplored, therapeutic target for SMA. Supported by Girotondo ONLUS, Smarathon ONLUS and University of Turin grants.

Disclosures: **M.M. Boido:** None. **V. Valsecchi:** None. **E. De Amicis:** None. **A. Piras:** None. **A. Vercelli:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS078429

MDA Grant 255293

Himelic Family Foundation

Neuroscience Graduate Interdisciplinary Program

ARCS Foundation

Title: TDP-43 influences synaptic function through translational regulation of synaptic mRNA targets

Authors: *A. COYNE, J. JOHANNESMEYER, D. ZARNESCU;
Univ. of Arizona, Tucson, AZ

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease affecting upper and lower motor neurons. TDP-43, an RNA binding protein linked to the majority of ALS cases, is involved in several aspects of RNA metabolism. Using a *Drosophila* model of ALS based on TDP-43 we have previously identified a role for TDP-43 in the translation regulation of specific mRNA targets. Here we use a combination of genetic, molecular, imaging, and electrophysiology approaches to show that TDP-43 regulates the translation of *hsc70* mRNA in a variant dependent manner. Hsc70 is a molecular chaperone that functions at multiple steps in the synaptic vesicle cycle. Overexpression of Hsc70 in the context of TDP-43 mitigates multiple aspects of TDP-43 toxicity including locomotor dysfunction and reduced lifespan. Notably, this rescue is dependent on the chaperone activity of Hsc70 as evidenced by genetic interactions with an Hsc70 ATPase mutant that no longer rescues TDP-43 dependent phenotypes. In addition, Hsc70 overexpression restores synaptic vesicle cycling, specifically in the context of disease associated mutant TDP-43. FM1-43 dye uptake experiments reveal defects in endocytosis and a reduction in the size of the readily releasable and recycling vesicle pools in both TDP-43 variants. However, upon overexpression of Hsc70, endocytosis is restored specifically only in the disease associated mutant. Furthermore, overexpression of Hsc70 reduces the amount of insoluble TDP-43 in a variant dependent manner. Our results provide evidence for TDP-43 regulating synaptic function and the synaptic vesicle cycle through the translational regulation of its synaptic mRNA targets. Ultimately, this dysregulation of translation may lead to depletion of key synaptic proteins at the neuromuscular junction and synaptic failure preceding neurodegeneration. Additionally, our results provide evidence for

differential regulation of mRNA targets by disease associated mutant TDP-43 and dysregulation of synaptic function through altered ribostasis, a key event in the progression of ALS.

Disclosures: **A. Coyne:** None. **J. Johannesmeyer:** None. **D. Zarnescu:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.07/O9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Northwestern Weinberg Grant (DH)

Les Turner ALS Foundation (PHO)

Wenske Foundation (PHO)

Title: Early connectivity defects of corticospinal motor neurons in ALS

Authors: *D. HELLER[1], J. H. JARA[1], P. SHEETS[2], M. NIGRO[2], M. MARTINA[2], G. M. G. SHEPHERD[2], P. H. OZDINLER[1,3,4];
[1]Neurol., [2]Physiol., Northwestern University, Feinberg Sch. of Med., Chicago, IL; [3]Northwestern University, Robert Lurie Cancer Ctr., Chicago, IL; [4]Northwestern University, Cognitive Neurol. and Alzheimer's Dis. Ctr., Chicago, IL

Abstract: Corticospinal motor neurons (CSMN) degenerate in various motor neuron disorders such as amyotrophic lateral sclerosis (ALS), primary lateral sclerosis, and hereditary spastic paraplegia. CSMN are long-distance projection neurons located in the layer V of the motor cortex that collect, integrate and transmit cerebral cortex' inputs toward spinal cord targets. Therefore, their proper modulation is essential for the initiation and modulation of voluntary movement. CSMN receive both excitatory and inhibitory inputs from many different neuron types in the cerebral cortex, and the fine balance between these excitatory and inhibitory inputs both from local circuitries and long-distance projection neurons modulate CSMN activity. We previously revealed a selective pattern of apical dendrite degeneration with spine loss especially in layer II/III of the $hSOD1^{G93A}$ ALS mouse motor cortex. This could account for early cellular CSMN vulnerability due their inability to process cerebral input. We developed an anatomical and electrophysiology approach to investigate mechanisms that could potentially be involved in CSMN vulnerability and progressive degeneration. Using electrophysiological recordings, coupled with retrograde labeling and analysis of distinct neurons types in different layers of the

motor cortex, we begin to reveal the details of early connectivity defects in ALS. Our ongoing studies suggest that CSMN degeneration could be due to a complex imbalance between excitation and inhibition signals, in addition to their intrinsic vulnerability.

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Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.08/O10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: National Science Foundation Graduate Research Fellowship Award

Graduate Program in Neuroscience at The Johns Hopkins University

Thomas Shortman Training Fund Graduate Scholarship

NIH

Target ALS

Robert Packard Center for ALS Research

MDA

Title: Aberrant nuclear pore pathology in c9orf72 als-ftd and neurodegeneration

Authors: *J. C. GRIMA[1], M. J. ELRICK[1], K. RUSSELL[1], L. PETRUCELLI[2], R. H. BROWN[3], L. W. OSTROW[1], C. J. DONNELLY[1], R. SATTLER[1], J. D. ROTHSTEIN[1];
[1]Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Mayo Clin., Jacksonville, FL; [3]Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Familial and sporadic Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) share a common genetic mutation in chromosome 9. An expanded hexanucleotide (GGGGCC) repeat within a non-coding region of the C9orf72 gene is the most common genetic cause of familial and sporadic ALS and FTD. It is also the most common cause of Huntington disease phenocopies. The repeat expansion leads to the loss of one alternatively spliced C9orf72 transcript, pathological inclusions of TDP-43 protein, the formation of nuclear

RNA foci and accumulation of cytoplasmic dipeptide repeats. However, the underlying mechanisms by which this bi-directionally transcribed expanded repeat causes these diseases have not been fully elucidated. Nonetheless, it is becoming increasingly evident that Nuclear Pore Complex (NPC) dysfunction may be a key pathogenic contributor. NPCs are the largest protein assemblies in eukaryotic cells. They span the nuclear envelope, consist of multiple copies of 30 different proteins called nucleoporins, and serve as the only transport conduit between the nucleus and cytoplasm. These molecular machines not only regulate the flow of molecules into and out of the nucleus but also have transport-independent functions such as regulating genome organization and gene expression. Work from our lab and others indicate that nucleocytoplasmic transport dysfunction may be a fundamental pathway for C9orf72 ALS-FTD pathogenesis. Proteins in this pathway are potent genetic modifiers of GGGGCC repeat expansion-mediated cytotoxicity in a *drosophila* model and in iPS neurons derived form C9orf72 patients. Our ongoing studies suggest that products of the C9orf72 repeat expansion are likely to disrupt nucleocytoplasmic transport at the NPC. To this end, we assessed the integrity of nucleoporins, the basic building blocks of NPCs, in C9orf72 human tissue, ips neurons, and various transgenic animal models. Our preliminary data using a large cohort of human autopsy brain and spinal cord as well as transgenic mouse tissues and human C9orf72 iPS cell lines indicate that select nucleoporins are severely affected in the disease with aggregation at the nuclear membrane and altered nuclear to cytoplasmic distribution. This study suggests NPC pathology and function are a fundamental defect in the pathway of C9orf72 ALS-FTD.

Disclosures: J.C. Grima: None. **M.J. Elrick:** None. **K. Russell:** None. **L. Petrucelli:** None. **R.H. Brown:** None. **L.W. Ostrow:** None. **C.J. Donnelly:** None. **R. Sattler:** None. **J.D. Rothstein:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.09/O11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Molecular classification of amyotrophic lateral sclerosis by unsupervised clustering of gene expression in motor cortex

Authors: G. MORELLO[1], E. ARONICA[2], F. BAAS[3], A. IYER[4], A. TEN ASBROEK[5], *S. CAVALLARO[1];
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Amsterdam, the Netherlands. Swammerdam Inst. for Life Sciences, Ctr. for Neuroscience; Univ. of Amsterdam, Amsterdam, The Netherlands., Amsterdam, Netherlands; [3]Neurogenetics,Academic Med. Ctr., Amsterdam, Netherlands; [4]Dept. of (Neuro) Pathology, Academic Med. Center, Amsterdam, the Netherlands., Amsterdam, Netherlands; [5]Dept. of Neurogenetics, Academic Med. Center, Amsterdam, the Netherlands., Amterdam, Netherlands

Abstract: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and ultimately fatal neurodegenerative disease, caused by the loss of motor neurons in the brain and spinal cord. Although 10% of ALS cases are familial (FALS), the majority are sporadic (SALS) and probably associated to a multifactorial etiology. Currently there is no cure or prevention for ALS. A prerequisite to formulating therapeutic strategies is gaining understanding of its etio-pathogenic mechanisms. In this study we analyzed whole-genome expression profiles of 41 motor cortex samples of control (10) and sporadic ALS (31) patients. Unsupervised hierarchical clustering was able to separate control from SALS patients. In addition, SALS patients were subdivided in two different groups that were associated to different deregulated pathways and genes, some of which were previously associated to familiar ALS. These experiments are the first to highlight the genomic heterogeneity of sporadic ALS and reveal new clues to its pathogenesis and potential therapeutic targets.

Disclosures: G. Morello: None. **E. Aronica:** None. **F. Baas:** None. **A. Iyer:** None. **A. ten Asbroek:** None. **S. Cavallaro:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.10/O12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Intramural Research Program of the National Institute on Aging

Title: TDP-43 accumulation causes aberrant motor neuron excitability

Authors: *Y. LIU[1], H. DONG[1,2], R. D. DAYTON[3], R. L. KLEIN[3], M. P. MATTSON[1]; [1]Lab. of Neurosci., Natl. Inst. on Aging, Baltimore, MD; [2]Dept. of Neurol., the Second Hosp. of Hebei Med. Univ., Shijiazhuang, Hebei, China; [3]Dept. of Pharmacology, Toxicology and Neurosci., Louisiana State Univ., Shreveport, LA

Abstract: TDP-43 (transactive response DNA-binding protein-43) accumulates in abnormally high amounts in motor neurons of patients with sporadic amyotrophic lateral sclerosis (ALS). Overexpression of TDP-43 result in TDP-43 accumulation and degeneration of motor neurons in animal models. To elucidate alterations in neuronal excitability that may occur in response to TDP-43 proteopathy prior to motor neuron degeneration, we performed whole-cell patch clamp recordings from motor neurons in spinal cord slices from mice overexpressing either wild type human TDP-43 (hTDP-43) or GFP. Expression in motor neurons was achieved using AAV-9 vectors that were injected intracerebroventricularly into neonatal mice (see Wang DB et al. Mol Ther 2010; 18:2064-2074). Recordings were made from spinal cord motor neurons of 7-10 day old pups, and tests of motor function were performed in 19-21 day old mice. Motor neurons of hTDP-43 mice exhibited hyperexcitability with a 37% greater frequency of spontaneous action potentials (AP), a lower threshold for AP induction and smaller AP amplitude compared to control mice. Motor neurons of hTDP-43 mice also showed more spikes during current step stimuli. Whereas control mice gained weight progressively, hTDP-43 mice lost weight beginning at day 14. By the time they were 19-21 days old hTDP-43 mice exhibited a greatly reduced grip strength and severe motor dysfunction on the rotarod. Our findings show that elevation of hTDP-43 levels results in hyperexcitability of motor neurons that occurs prior to motor deficits and neuronal death. We are currently developing interventions to counteract the adverse effects of hTDP43 on motor neuron excitability and degeneration.

Disclosures: Y. Liu: None. **H. Dong:** None. **R.D. Dayton:** None. **R.L. Klein:** None. **M.P. Mattson:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.11/O13

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS051419

NIH Grant NS062055

Muscular Dystrophy Association

Title: The role of mitophagy and parkin in SOD1-ALS

Authors: G. M. PALOMO[1], A. ARREGUIN[1], J. MAGRANE[1], *G. MANFREDI[2];
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[2]Weill Med. Col. Cornell Univ., New York, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease caused by the degeneration of upper and lower motor neurons. Familial forms represent 10% of all cases, 20% of which are linked to mutations in the Cu-Zn superoxide dismutase 1 (SOD1) gene. Different mechanisms have been proposed to explain the deleterious effects of mutant SOD1, including accumulation of misfolded protein on the mitochondrial outer membrane and in the intermembrane space and mitochondrial structural and functional damage, suggesting that mitochondrial dysfunction plays a role in disease pathogenesis. Damaged mitochondria need to be either repaired or eliminated by quality control mechanisms to maintain a sufficient pool of functional mitochondria in neurons. Selective mitochondrial autophagy (mitophagy) is essential to this process. Here, we investigated mitophagy in G93A mutant SOD1. We used spinal cords of G93A-SOD1 transgenic mice and motor neuron-like NSC34 cells, stably transfected with either mitochondria-targeted SOD1 or untargeted SOD1. We studied the involvement of several key players of autophagy, mitophagy, and mitochondrial dynamics. In G93A spinal cords, Parkin, an E3 ubiquitin ligase involved in initiation of mitophagy, was decreased at all disease stages tested (pre-symptomatic, disease onset and end stage). However, Parkin mRNA levels were not affected, suggesting that the decline of the protein was due to increased turnover. Similarly, Drp-1, a protein necessary for mitochondrial fission, was reduced in G93A mutant spinal cords, at disease onset and end stage. Furthermore, we detected increases in ubiquitination of mitochondrial proteins by western blot, using both lysine 63 and lysine 48 ubiquitin antibodies, and in the association of p62, an adaptor with the autophagosomes, with mitochondrial fractions. NSC34 cells expressing mutant or wild type SOD1 were induced to differentiate and transfected with YFP-Parkin (NSC34 do not express detectable endogenous Parkin), prior to exposure to stress by removal of antioxidants from the culture media. Parkin expression was protective, suggesting that facilitating mitophagy through Parkin overexpression may contribute to neuroprotection in SOD1 mutant cells. Together, these results suggest a mechanism by which excessive requirements for the removal of dysfunctional and damaged mitochondria in mutant SOD1 neurons lead to exhaustion of essential components of mitophagy, such as Parkin and Drp-1 and to the accumulation of ubiquitin-tagged mitochondria. Inability to maintain an adequate mitochondria quality control could contribute to the motor neuron demise in ALS.

Disclosures: G.M. Palomo: None. **A. Arreguin:** None. **J. Magrane:** None. **G. Manfredi:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.12/O14

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Les Turner ALS Foundation

Wenske Foundation

Brain Research Foundation

5T32AG020506-10

NIH-R21 NS085750-01

Title: AlsinKO-UeGFP mice, the CSMN reporter line for Alsin, display CSMN-specific cellular defects without major cell loss

Authors: *M. GAUTAM[1], G. SEKERKOVA[2], J. H. JARA[1], M. V. YASVOINA[1], H.-X. DENG[1], M. MARTINA[2], P. H. ÖZDINLER[3];
[1]Neurol., Dept. of Neurology, Northwestern Univ., Chicago, IL; [2]Physiol., [3]Neurol., Northwestern Univ., Chicago, IL

Abstract: Corticospinal motor neurons (CSMN) are unique in their ability to collect, integrate, translate and transmit cerebral cortex's input towards spinal cord targets. Their degeneration is the key in numerous neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS). Mutations in the Alsin 2 (ALS2) gene are reported to be responsible for juvenile primary lateral sclerosis, infantile onset ascending hereditary spastic paraplegia, and are the most common cause for autosomal recessive juvenile ALS. In addition, upper motor neuron signs and bulbar symptoms are often prevalent in patients with juvenile ALS. However, cellular and molecular aspects of CSMN degeneration has not been studied in detail due to lack of selective markers to visualize these neuron populations *in vivo*. By crossing UCHL1-eGFP with AlsinKO, we generated AlsinKO-UeGFP mice, a CSMN reporter line to investigate upper motor neuron defects in the absence of Alsin. This novel reporter line helped us visualize and study CSMN at different stages of disease progression. Different from the hSOD1G93A mice or the TDP-43 mouse models, the numbers of CSMN do not show dramatic reduction in the absence of Alsin. However, detailed cellular analysis using immunocytochemistry coupled with electronmicroscopy (EM) revealed very precise aspects of cellular defects that are restricted to CSMN. We find that even though CSMN do not undergo massive cell loss, the neurons are not healthy. The apical dendrites of CSMN become vacuolated, and this cellular defect is observed only in CSMN in the motor cortex. In addition, there are defects in the mitochondria, and there are signs of defective autophagy, with enlarged lysosomes that contain defective mitochondria,

in addition to other proteins. The integrity of the cell membrane is impaired and becomes leaky especially towards end-stage. These findings suggest that Alsin is an important protein for proper CSMN function, and in its absence CSMN display precise neuronal defects, but such defects do not initiate their clearance. Therefore, even though the neurons are still present at layer V of the motor cortex, they are unhealthy and potentially nonfunctional.

Disclosures: **M. Gautam:** None. **G. Sekerkova:** None. **J.H. Jara:** None. **M.V. Yasvoina:** None. **H. Deng:** None. **M. Martina:** None. **P.H. Özdinler:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Department of Defense W81XWH-11-1-0689 (GZM)

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NIH R21NS077038 (LP)

NIH R01NS069601 (LP)

Title: Expression of Stasimon rescues select defects in the sensory-motor circuit of SMA mice

Authors: *C. M. SIMON, F. LOTTI, E. BIANCHETTI, S. TISDALE, G. Z. MENTIS, L. PELLIZZONI;
Col. of Physicians and Surgeons, Motor Neuron Ctr., New York, NY

Abstract: Ubiquitous SMN deficiency causes the neurodegenerative disease SMA. While SMN activity in the assembly of spliceosomal snRNPs is well established, splicing targets of SMN deficiency that contribute to motor system pathology in mouse models of SMA are unknown. We previously identified Stasimon as an evolutionarily conserved, SMN-regulated U12 intron-containing gene that contributes to motor circuit deficits induced by SMN depletion in *Drosophila* and zebrafish models. Here, we investigated the role of Stasimon dysfunction in an SMA mouse model. We found that SMN deficiency disrupts U12 splicing and reduces expression of Stasimon mRNA in motor circuit neurons of SMA mice. To study the effects of increased Stasimon expression on the disease phenotype of SMA-Δ7 mice, we employed ICV injection of scAAV9 vectors at P1, which results in efficient transduction of motor neurons and

sensory neurons. scAAV9-GFP and scAAV9-SMN were used as negative and positive controls, respectively. Stasimon restoration neither enhanced SMN expression nor corrected snRNA levels and downstream RNA processing events disrupted by SMN deficiency in SMA mice, consistent with Stasimon being a downstream mRNA target of SMN function in splicing. Importantly, Stasimon expression improved motor behavior of SMA mice assessed by righting time. Remarkably, while Stasimon did not improve neuromuscular junction denervation of affected muscle, it rescued the loss of proprioceptive synapses on both soma and dendrites of motor neurons. Moreover, functional assays revealed robust, Stasimon-dependent functional improvement of central sensory-motor neurotransmission, which is severely disrupted in SMA mice. Correction of these morphological and functional abnormalities of the motor circuit was associated with reduced loss of SMA motor neurons. Our work reveals the direct contribution of Stasimon dysfunction to select deficits in the sensory-motor circuit of SMA mice, establishing Stasimon as the first RNA splicing target of SMN deficiency with a demonstrated role in SMA pathology of an animal model that resembles the human disease. Our findings that Stasimon restoration improves some but not all of the disease-related deficits in SMA mice also support the view that SMA pathology results from a complex set of defects involving SMN-dependent mis-splicing of multiple genes and possibly disruption of other RNA pathways. Lastly, our results identify Stasimon as a gene with a key role in development and disease of the mammalian motor system.

Disclosures: **C.M. Simon:** None. **F. Lotti:** None. **E. Bianchetti:** None. **S. Tisdale:** None. **G.Z. Mentis:** None. **L. Pellizzoni:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.14/O16

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR

Title: Activation of the sigma-1 receptor (Sig-1R) by ligands or ER stress results in increased receptor mobility, subcellular redistribution and the modulation of the Unfolded Protein Response

Authors: ***A. Y. WONG**[1], P. CHUDALAYANDI[1], E. HRISTOVA[1], N. AHLSKOG[1], J. K. NGSEE[2,1], R. BERGERON[1,2];

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Abstract: Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by the selective degeneration of upper and lower motor neurons (MNs). While an array of triggers exists for ALS, they all converge on one of two main pathological mechanisms, RNA metabolism or proteostasis. One form of familial ALS (ALS 16) arises from a point mutation (E102Q) in the ligand-binding domain of an ER chaperone protein, the sigma-1 receptor (Sig-1R). As the Sig-1R is a protein chaperone, it is thought to modulate proteostasis during endoplasmic reticulum (ER) stress by modulating the Unfolded Protein Response (UPR), which is upregulated in ALS and could contribute to MN degeneration. **Results:** Induction of ER stress, by either DTT or Tunicamycin, resulted in an increased mobility of wild-type (WT) Sig-1R, and aggregation into immobile puncta. Co-localization analysis suggests that these puncta are ER quality control compartments. In contrast, the E102Q mutant has a significantly higher mobility and tendency to cluster in the absence of ER stress. Activation of wild-type Sig-1R by the agonist SKF-10,047 attenuated the expression of apoptotic markers and promoted recovery from DTT-induced ER stress in NSC-34 cells and MEFs derived from WT mice. This was not observed in NSC-34 overexpressing E102Q or MEFs from Sig-1R$^{-/-}$ knockout mice. **Conclusion:** Aggregation of the E102Q mutant leads to dysregulation of adaptive mechanisms of the UPR during ER stress and subsequent MN death in ALS.

Disclosures: A.Y. Wong: None. **P. Chudalayandi:** None. **E. Hristova:** None. **N. Ahlskog:** None. **J.K. Ngsee:** None. **R. Bergeron:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.15/O17

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: IWT grant 131535

Title: Expression of human FUS induces an early loss of CCAP neurons resulting in wing expansion and cuticle tanning defects in *Drosophila*

Authors: J. A. STEYAERT[1], N. WILMANS[1], W. SCHEVENEELS[1], P. VAN DAMME[2], W. L. ROBBERECHT[2], P. CALLAERTS[3], E. BOGAERT[1], *L. M. VAN DEN BOSCH[1]; [1]Lab. of Neurobiology, Vesalius Res. Center, VIB, Leuven, Belgium, Leuven, Belgium; [2]Univ.

Hosp. Leuven, Dept. of Neurol., Leuven, Belgium; [3]Lab. of Behavioral and Developmental Genetics, VIB, KU Leuven, Leuven, Belgium

Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) belong to a group of complex neurodegenerative disorders. A subgroup within this disease spectrum, the FUSopathy, is characterized by FUS inclusions in neurons and glial cells. The observation that mutations in FUS cause ALS emphasizes the involvement of FUS in the disease pathogenesis. In order to examine the pathogenic role of FUS, we generated four different transgenic fly lines, allowing expression of wild type human FUS (WT hFUS) and three disease-associated mutant human FUS proteins (R521G hFUS, R521H hFUS and P525L hFUS). Selective expression of hFUS transgenes in the motor neurons via the UAS-GAL4 expression system resulted in a partial developmental lethality as pharate adults were not able to hatch. Furthermore, adult escapers show a wing inflation phenotype and a defect in cuticle tanning. These defects are posteclosion events regulated by the secretion of the neurohormone 'Bursicon' by a subset of 15 'crustacean cardioactive peptide' (CCAP) expressing neurons located in the ventral nerve cord. Restricting hFUS expression to these neurons recapitulated partially the phenotype: the posteclosion defects were still present, but the pupal lethality was absent. Toxicity, induced by mutant hFUS expression in the CCAP neurons, causes an accelerated cell death. Furthermore, this cell death is most pronounced in the CCAP neurons that secrete Bursicon, although selective hFUS expression in these neurons is not toxic. We hypothesize that overall toxicity in all CCAP neurons is needed to affect Bursicon secretion. To prove this, Bursicon and CCAP levels will be analysed in the hemolymph. In conclusion, our findings indicate that mutant human FUS expression in CCAP neurons is the underlying cause of the immature fly phenotype which is caused by neuronal death of a subset of neurons.

Disclosures: **J.A. Steyaert:** None. **N. Wilmans:** None. **W. Scheveneels:** None. **P. Van Damme:** None. **W.L. Robberecht:** None. **P. Callaerts:** None. **E. Bogaert:** None. **L.M. Van Den Bosch:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.16/O18

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NRF 2014R1A1A4A01003859

Korean Health Technology R&D Project, Ministry for Health, Welfare &Family Affairs, Republic of Korea HI14C1891

Title: Cellular localization of ALS-linked FUS regulates neurite fragmentation under oxidative stress

Authors: M.-H. JUN, H.-H. RYU, W.-K. JEONG, Y.-K. LEE, *J.-A. LEE;
Hannam Univ., Dajeon, Korea, Republic of

Abstract: Mutations in fused in sarcoma (FUS), a DNA/RNA binding protein, are associated with familial amyotrophic lateral sclerosis (fALS) and some forms of frontotemporal lobar dementia (FTLD). However, little is known about how specific ALS-causing mutations induce neurodegeneration associated with FUS positive inclusion and stress granules. In this study, we identified protein arginine methyltransferase 1 (PRMT1) as an associating protein with ALS-linked FUS (R521C). Association between R521C and PRMT1 was RNA dependent, but methylation independent. Loss of PRMT1 cause accumulation of FUS-positive stress granules and neurite fragmentation under oxidative stress while overexpression of PRMT1 reduce cytosolic localization of FUS into stress granule and neurite fragmentation in FUS(R521C) expressing neurons. Taken together, these data suggest that PRMT1 could regulate FUS-associated SGs and neurite fragmentation under oxidative stress. Overall, this study provide a novel pathogenic mechanism of the FUS mutation associated with oxidative stress in ALS and therapeutic insight regarding FUS pathology

Disclosures: M. Jun: None. **H. Ryu:** None. **W. Jeong:** None. **Y. Lee:** None. **J. Lee:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.17/O19

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NHMRC APP1065884

MNDRIA Grant-in-Aid GIA1403

Title: The respiratory motor system in amyotrophic lateral sclerosis - dendritic growth and regression is coupled with motor neuron loss and impaired respiratory function

Authors: *M. C. BELLINGHAM[1], M. J. FOGARTY[2], E. W. H. MU[2], J. R. DRIEBERG-THOMPSON[2], N. A. LAVIDIS[2], P. G. NOAKES[2,3];
[2]Sch. of Biomed. Sci., [3]Queensland Brain Inst., [1]Univ. of Queensland, Brisbane, Australia

Abstract: The progressive loss of respiratory motoneurons (MN) in brainstem and spinal cord is a critical factor in respiratory impairment and death in amyotrophic lateral sclerosis (ALS). However, while evidence suggests that MN intrinsic excitability and excitatory synaptic inputs onto MNs are increased prior to MN death, the timing and nature of changes in the respiratory motor system remains unclear. In pre-symptomatic (postnatal [P] day 25-35), disease onset (P60-70), mid-disease (P100-120) and end-stage (>P140) $hSOD1^{G93A}$ (SOD1) and wild type (WT) control mice, we examined single respiratory MN morphology, using Golgi-Cox impregnation for brainstem hypoglossal (XII) MNs or retrograde labelling with Alexa555-cholera toxin B for spinal cord phrenic MNs (n=10 animals per age/genotype). MN arbors were traced in Neurolucida and were analysed by two-way ANOVA with Bonferroni post-tests, with significance at $p<0.05$. Unbiased stereological counts of XII and phrenic MNs counts were done (n=>4 animals per age/genotype). Unrestrained whole body plethysmography (Buxco) measured respiration volumes and frequency from P125 onwards (n=8 per genotype). Progressive increases in dendritic arbors (branch elongation and more branching) of XII (49%) and phrenic MNs (63%) were seen at pre-symptomatic ages in SOD1 mice compared to WT mice. Pre-symptomatic XII MNs had a 51% increase in dendritic spine density/100 μm dendrite length. XII MNs showed a 51% decrease in dendrite arbors at disease onset age, while phrenic MN dendrite arbours had regressed and were not significantly different to WT phrenic MN arbors. Both XII (-74%) and phrenic (-20%) MNs showed reduction in dendritic arbors at mid-disease ages. XII MN numbers decreased from P35 onward, while phrenic MN numbers did not decrease until mid-disease age. Respiratory volumes but not frequency were significantly decreased in SOD1 mice from P125. Our results suggest that significant morphological changes, illustrated by excessive arborisation and spine increases, occur prior to loss of key respiratory MNs, while respiratory MN loss is associated with dendrite regression and impaired respiratory function. Interestingly, respiratory MNs seem to be degenerating at a slower rate or later age, compared to lumbar MNs. Pre-symptomatic structural changes seen in respiratory MNs may represent an adaptive response, which reduces or delays respiratory dysfunction. Our findings correlate with symptom progression seen in many ALS patients, where declines in respiratory motor function are typically seen later in disease progression.

Disclosures: M.C. Bellingham: None. **M.J. Fogarty:** None. **E.W.H. Mu:** None. **J.R. Drieberg-Thompson:** None. **N.A. Lavidis:** None. **P.G. Noakes:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.18/O20

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant 1R01NS078214

Title: Active zone degeneration causes neuromuscular junction denervation in ALS model rodents

Authors: *H. NISHIMUNE[1], S. TUNGTUR[1], J. A. STANFORD[2], T. TANAKA[1], L. L. NADEAU[1];
[1]Anat. & Cell Biol., [2]Mol. & Integrative Physiol., Univ. of Kansas Sch. of Med., Kansas City, KS

Abstract: Amyotrophic lateral sclerosis (ALS) model rodents exhibit denervation of neuromuscular junctions (NMJs) prior to motor neuron degeneration, suggesting that ALS is a dying-back neuropathy. However, etiology of NMJ denervation and ALS remains unknown. To investigate the cause of NMJ denervation in ALS, we focused on NMJ active zones, which are essential presynaptic structure for synaptic transmission. Active zone loss impairs synaptic transmission efficiency. We have analyzed the numbers of active zones and active zones aligned with junctional folds in innervated NMJs of $SOD1^{G93A}$ male mice using electron microscopy, and compared them to age- and sex-matched wild-type mice. Active zone number decreased in the presymptomatic stage before significant denervation was detected in diaphragms. However, NMJ size and total number of synaptic vesicle in nerve terminals did not change between $SOD1^{G93A}$ and wild-type mice. Degenerated mitochondria were detected in presynaptic terminals, but active zone defect did not correlate with the detection of degenerated mitochondria. These results suggest that the active zone loss is a very early defect of ALS NMJs, which is not a secondary defect to degeneration of presynaptic terminals or mitochondria degeneration. The causality of active zone loss in NMJ denervation was tested using a mouse model of active zone loss. Previously, we identified a molecular mechanism for active zone organization, which is accomplished by a synapse organizer laminin β2 binding extracellularly to its specific receptor P/Q-type voltage dependent calcium channels and anchoring active zone proteins to presynaptic membrane including Bassoon. Acute inhibition of the interaction between the ligand laminin β2 and its receptor calcium channels causes a decrease of active zone number in NMJs of wild type mice. In the current study, the laminin β2 - calcium channel interaction was chronically inhibited, which caused significant denervation of NMJs in wild-type mice. These data suggest that a decrease of active zone number causes NMJ denervation in ALS. Interestingly, the NMJ denervation can be ameliorated by exercise indicating a potential intervention to ameliorate the early stage ALS symptom. In summary, active zone degeneration plays a key role in NMJ denervation in ALS model rodents.

Disclosures: **H. Nishimune:** None. **S. Tungtur:** None. **J.A. Stanford:** None. **T. Tanaka:** None. **L.L. Nadeau:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.19/O21

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: GoBio Grant

Title: Immortalized human neuronal progenitor cells (ReNcell CX) are an ideal model to investigate the inhibition of adult neurogenesis in the SVZ mediated by TGF-β

Authors: *S. KUESPERT[1], E. ZITZELSPERGER[1], S. PETERS[1], S. KLATT[2], T.-H. BRUUN[1], L. AIGNER[3], U. BOGDAHN[1];
[1]Dept. of Neurol., [2]Dept. of Hematology, Univ. Hosp. Regensburg, Regensburg, Germany; [3]Inst. Regenerative Med., Paracelsus Med. Univ., Salzburg, Austria

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative lethal disorder with no effective treatment so far. The current molecular genetic campaign is increasingly elucidating the molecular pathogenesis of this fatal disease; from previous studies it is known that Transforming Growth Factor-β (TGF-β) is found in high concentrations in Cerebrospinal Fluid (CSF) (Morganti-Kossmann et al., 1999) of ALS patients. These high levels of circulating TGF-β are known to promote stem cell quiescence and therefore lead to inhibition of adult neurogenesis within the sub ventricular zone (SVZ) of the brain (Kandasamy et al., 2014). Thus, regeneration of degenerating neurons seems to be prevented by an enhanced TGF-β signaling. In this study we wanted to figure out if selective inhibition of TGF-β signaling mediated by antisense-oligonucleotide against TGF-βRII might allow regeneration of adult neurogenesis. First we investigated if an immortalized human neuronal progenitor cell line isolated from the cortical region of human fetal brain (ReNcell CX; Millipore) is a proper model to analyze the TGF-β-mediated effects. Therefore, we examined the ability of ReNcell CX to respond to TGF-β by analyzing the respective molecules up- and downstream (TGF-β1, 2 and 3, pSmad2/3, CTGF, PAI-1) of the canonical TGF-β pathway by quantitative RT-PCR, immunoblotting and immunocytochemistry. Next, stem cell quiescence induced by TGF-β1 is shown on ReNcell CX cells by treatment of the cells with 10 or 50 ng/ml TGF-β1 for 7 d. Afterwards, ReNcell CX cells were treated with different concentrations (2.5 and 10 µM) of a TGF-βRII - antisense-oligonucleotide by gymnotic delivery for different time points. Probes were subsequently

examined with regard to downregulation of TGF-βRI and II and its effect on Smad2/3-cascade, MAP/Erk- and Akt-pathway by quantitative RT-PCR, immunoblotting and immunocytochemistry. Results indicate that ReNcell CX cells represent an adequate model to investigate regeneration of adult neurogenesis by selective antisense oligonucleotide-mediated blocking of TGF-β signaling due to TGF-β response, cell cycle arrest following TGF-β-exposure. Further, the ReNcell CX cell line demonstrated a proper downregulation of TGF-βRII and showed thereby an inhibition of TGF-β signaling due to gymnotic transfer of a selective antisense-oligonucleotide against TGF-βRII.

Disclosures: **S. Kuespert:** None. **E. Zitzelsperger:** None. **S. Peters:** None. **S. Klatt:** None. **T. Bruun:** None. **L. Aigner:** None. **U. Bogdahn:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.20/O22

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant RO1HD64850

FightSMA Emerging Investiagor Award

CureSMA Audrey Lewis Young Investigator Award

Title: Reciprocal binding between SMN and the COPI coatomer subunit alpha-COP is required for neuronal morphology in cell and animal models of spinal muscular atrophy

Authors: ***S. K. CUSTER**[1], H. LI[1], L. T. HAO[2], C. E. BEATTIE[2], E. J. ANDROPHY[1]; [1]Dermatol., Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Spinal muscular atrophy (SMA) is a hereditary neuromuscular disorder caused by loss of the SMN1 gene and the resultant decrease in its protein product, Survival Motor Neuron (SMN). The functions of the SMN protein in developing and mature neurons remain a mystery. We are using cell and animal model systems to understand the importance of the SMN interaction with the alpha-COP subunit of the COPI vesicle. Although mainly involved in Golgi-ER retrograde trafficking, the α-COP coat protein is observed in cultured neurons in the Golgi, growth cone, and neurite, and we have reported that SMN moves with α-COP in these neuronal processes. To characterize the biological role of the association between SMN and α-COP, we

mutated a dilysine motif in SMN exon 2b, which we previously identified as the minimal α-COP binding domain in SMN, and found these do not bind to α-COP and are unable to restore neurite outgrowth in our NSC-34 cells model of SMA. We have now been able to demonstrate the functional significance of the interaction of SMN with α-COP *in vivo* using murine and zebrafish models of SMA. Surprisingly, transient expression of α-COP in SMN-depleted NSC-34 cultures restores neurite outgrowth without increasing SMN protein levels. We have also queried the functions of alpha-COP in developing neuronal cells. α-COP knockdown completely abolished the ability of NSC-34 cells to form neurites, which was restored by transient expression of human α-COP. Depletion of α-COP in primary cortical neurons showed a similar effect on both Map2 positive dendrites and tau positive axons. We have isolated a point mutant of α-COP which is unable to bind SMN, but retains the ability to form COPI coatomer as evidenced by immunoprecipitation of the epsilon, beta and gamma COP subunits. This mutant α-COP is unable to restore neurite outgrowth in α-COP depleted NSC-34 cells despite the fact that it rescue normal Golgi-ER retrograde traffic. This mutant was unable to restore neurite outgrowth in SMN-depleted NSC-34 cultures, demonstrating that there is reciprocal functional importance for the SMN/ α-COP interaction in the growth and maintenance of neuronal processes in normal conditions and SMA model cells. Finally, RNA from the dilysine SMN mutants, wildtype and mutant α-COP were injected into SMN depleted zebrafish embryos. Wildtype SMN fully restored motor neuron pathfinding, while the dilysine mutants did not. Similar to what we've seen in our NSC-34 cell model of SMA, wildtype α-COP significantly restored motor neuron morphology while the mutant which is unable to bind SMN did not. These data demonstrated that this interaction is crucial for proper growth of motor neurons in a vertebrate animal model of SMA

Disclosures: **S.K. Custer:** None. **H. Li:** None. **L.T. Hao:** None. **C.E. Beattie:** None. **E.J. Androphy:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.21/O23

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Muscular Dystrophy Association

ALS Association

NIH Grant NS078429

Title: Poly(A)-binding protein nuclear 1 suppresses TDP-43 toxicity and aggregation in ALS disease models

Authors: *C.-C. CHOU[1,2], O. M. ALEXEEVA[1], Y. ZHANG[1,5], F. LIU[1,6], B. MO[1], K. R. WILLIAMS[1], D. C. ZARNESCU[7], N. T. SEYFRIED[3,4,2], G. J. BASSELL[1,2,4], W. ROSSOLL[1,2]; [1]Dept. of Cell Biol., [2]Ctr. for Neurodegenerative Dis., [3]Dept. of Biochem., [4]Dept. of Neurol., Emory Univ., Atlanta, GA; [5]Dept. of Neurol., Xiangya Hospital, Central South Univ., Changsha, Hunan, China; [6]Dept. of Ophthalmology, the Second Hosp. of Jilin Univ., Changchun, China; [7]Dept. of Mol. and Cell. Biol., Univ. of Arizona, Tucson, AZ

Abstract: Among the growing number of known amyotrophic lateral sclerosis (ALS) disease proteins, TAR DNA binding protein 43 (TDP-43) has emerged as a key player. Nearly all sporadic and familial ALS cases are characterized by cytoplasmic aggregations of hyper-phosphorylated, ubiquitinated, and cleaved TDP-43 fragments and the loss of nuclear TDP-43. TDP-43 pathology is also common in frontotemporal dementia (FTD-TDP), as well as other TDP-43 proteinopathies. In rare cases, mutant TDP-43 can trigger the ALS disease process, establishing a clear causal role for TDP-43 in neurodegeneration. TDP-43 inclusion pathology may reflect an exaggeration of normal accumulation of TDP-43 into cytoplasmic RNA granules under stress conditions. The toxic aggregation in the cytoplasm as well as the loss of endogenous TDP-43 from the nucleus may contribute to the disease progression by impairing normal RNA and protein homeostasis. Therefore, both the removal of pathological protein and the rescue of TDP-43 mislocalization may be critical for halting or reversing TDP-43 proteinopathies. In this study, we investigated the effect of pathogenic TDP-43 and regulators of TDP-43 toxicity in *in vivo* and *in vitro* models. From a yeast-two-hybrid screen and co-immunoprecipitation experiments, we identified poly(A)-binding protein nuclear 1 (PABPN1) as a novel TDP-43 interaction partner that acts a potent suppressor of TDP-43 toxicity in yeast, *Drosophila*, and cultured primary neurons. PABPN1 expression reduced TDP-43 aggregation and also restored the nuclear localization of endogenous TDP-43, while promoting the turnover of disease-associated mutant TDP-43 protein and its C-terminal fragment. Quantitative proteomics suggested that PABPN1 rescued the perturbation of protein homeostasis in TDP-43 pathology. In addition, PABPN1 expression rescued the dysregulation of SG dynamics observed in our cellular models of TDP-43 proteinopathy. Knock-down of PABPN1 on the other hand enhanced TDP-43 aggregation and mislocalization and reduced cell viability. Taken together, these findings demonstrate a role for PABPN1 in rescuing several cytopathological features of TDP-43 proteinopathy by selectively increasing the turnover of pathogenic proteins.

Disclosures: C. Chou: None. **O.M. Alexeeva:** None. **Y. Zhang:** None. **F. Liu:** None. **B. Mo:** None. **K.R. Williams:** None. **D.C. Zarnescu:** None. **N.T. Seyfried:** None. **G.J. Bassell:** None. **W. Rossoll:** None.

Poster

068. Motoneuron Disease: Cellular Mechanisms I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 68.22/O24

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NRF Grant (2006-0093855)

NRF Grant (2014R1A1A1002076)

NRF Grant (2014R1A1A3052540)

Title: Iron accumulation promotes TACE-mediated TNF-α secretion and neurodegeneration in a mouse model of ALS

Authors: *J.-K. LEE[1], J.-H. SHIN[2], B. GWAG[3], E.-J. CHOI[1];
[1]Korea Univ., Seoul, Korea, Republic of; [2]Sch. of Med., Ajou Univ., Suwon, Korea, Republic of; [3]GNT Pharma, Suwon, Korea, Republic of

Abstract: Oxidative stress contributes to degeneration of motor neurons in patients with amyotrophic lateral sclerosis (ALS) as well as transgenic mice overexpressing ALS-associated human superoxide dismutase 1 (SOD1) mutants. However, the molecular mechanism by which the ALS-linked SOD1 mutants including SOD1(G93A) induce oxidative stress remains unclear. Here, we show that iron was accumulated in ventral motor neurons from SOD1(G93A)-transgenic mice even at 4 weeks of age, subsequently inducing oxidative stress. Iron chelation with deferoxamine mesylate delayed disease onset and extended lifespan of SOD1(G93A) mice. Furthermore, SOD1(G93A)-induced iron accumulation mediated the increase in the enzymatic activity of TNF-α converting enzyme (TACE), leading to secretion of TNF-α at least in part through iron-dependent oxidative stress. Our findings suggest iron as a key determinant of early motor neuron degeneration as well as proinflammatory responses at symptomatic stage in SOD1(G93A) mice.

Disclosures: J. Lee: None. **J. Shin:** None. **B. Gwag:** None. **E. Choi:** None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.01/O25

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Monocyte subtypes in ALS

Authors: *L. J. ZONDLER[1], S. KHALAJI[2], K. MUELLER[1], C. BLIEDERHAEUSER[1], V. GROZDANOV[1], W. P. RUF[1], A. FREISCHMIDT[1], P. WEYDT[1], K. E. GOTTSCHALK[2], A. C. LUDOLPH[1], K. M. DANZER[1], J. H. WEISHAUPT[1];
[1]experimental Neurol., [2]Dept. of experimental Physics, Ulm Univ., Ulm, Germany

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is characterized by progressive loss of motor neurons. The underlying pathogenic mechanisms of ALS are multifactorial and, at present, not fully determined, therefore the treatment options for ALS patients are very limited. Neuronal loss in ALS is accompanied by a neuroinflammatory reaction including proliferation of CNS resident microglia, increased cytokine levels in the blood and CNS and recruitment of peripheral immune cells to the CNS. While the infiltration of lymphocytes to the CNS of ALS patients has been described before, we show for the first time that peripheral monocytes also invade the CNS of ALS patients. Moreover, we found a significant redistribution of monocytic subpopulations in the peripheral blood of ALS patients, and pre-symptomatic carriers of ALS mutations. Peripheral human monocytes are dividable into two subpopulations, the pro-inflammatory CD14++ monocytes and the regenerative CD16++ monocyte subtype. We show, that the pro-inflammatory CD14++ monocyte subpopulation is significantly overrepresented in the blood of ALS patients and pre-symptomatic carriers of ALS mutations compared to healthy, age-matched controls, shifting the monocytic composition towards the pro-inflammatory side. Functional characterization of the CD14++ monocyte subpopulation revealed impaired phagocytosis and vesicle trafficking in CD14++ monocytes from sALS patients, as well as altered adhesive properties and migration potential. Concordantly, we found CD14++ monocytes of sALS patients to exhibit a distinct gene expression signature, not only separating sALS monocytes from monocytes of healthy donors, but also substantiating the functional differences described above. Further, we found that immunomodulatory treatment of ALS patient derived CD14++ monocytes *in vitro* reverses the shift of monocyte subpopulations observed in ALS patients and delays the disease onset in an ALS mouse model *in vivo*. Taken together, our findings indicate the involvement of especially the CD14++ monocyte subpopulation in ALS and we present a possible strategy of pharmacological intervention.

Disclosures: L.J. Zondler: None. **S. Khalaji:** None. **K. Mueller:** None. **C. Bliederhaeuser:** None. **V. Grozdanov:** None. **W.P. Ruf:** None. **A. Freischmidt:** None. **P. Weydt:** None. **K.E. Gottschalk:** None. **A.C. Ludolph:** None. **K.M. Danzer:** None. **J.H. Weishaupt:** None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.02/O26

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Cell-to-cell transmission of TDP-43 across axon terminals

Authors: *M. FEILER[1], B. STROBEL[3], A. FREISCHMIDT[2], A. HELFERICH[2], B. BREWER[4], D. LI[4], D. THAL[2], A. C. LUDOLPH[2], K. M. DANZER[2], J. H. WEISHAUPT[2]; [1]Experimenal Neurol., [2]Ulm Univ., Ulm, Germany; [3]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany; [4]Vanderbilt Univ., Nashville, TN

Abstract: Causative mutations in TDP-43-encoding gene TARDBP were identified in fALS and sALS patients. Furthermore, TDP-43 is an aggregation-prone prion-like domain-containing protein and component of pathological intracellular aggregates. TDP-43 oligomers have been postulated to be released and subsequently nucleate TDP-43 oligomerization in recipient cells, which might be the molecular correlate of the systematic symptom spreading observed during ALS progression. We developed a novel protein complementation assay allowing quantification of TDP-43 oligomers in living cells. This assay is based on the principle of using non-bioluminescent Gaussia princeps luciferase fragments fused to TDP-43. Aggregation of the TDP-43 molecules leads to reassembly of the two luciferase fragments - complementation - and therefore luciferase activity. Initial assay validation studies not only demonstrated that luciferase signal is a specific result of TDP-43 oligomerization and not promoted by potential self-complementation of the luciferase fragments, but also prove that cellular stress-induced TDP-43 aggregation can, as expected, be detected by the assay. Therefore, we used the assay to investigate TDP-43 oligomerization and axonal transport. Our results show that mutated TDP-43 has a higher tendency to aggregate as compared to the wild type protein. Moreover, we demonstrate the presence of TDP-43 oligomers in microvesicles/exosomes and show that microvesicular TDP-43 is preferentially taken up by recipient cells, where it exerts higher toxicity than free TDP-43.Using nanotechnology-derived microfluidic devices that fluidically isolate neuronal cell bodies from axon terminals in culture, we show both, anterograde and retrograde trans-synaptic transmission of TDP-43. Finally, we demonstrate TDP-43 oligomer seeding by TDP-43 derived from both, cultured cells and ALS patient-derived CNS tissue extracts. Thus, using an innovative detection technique, we provide evidence for preferentially microvesicular and bidirectional synaptic intercellular transmission and seeding ability of TDP-43 oligomers.

Disclosures: **M. Feiler:** None. **B. Strobel:** None. **A. Freischmidt:** None. **A. Helferich:** None. **B. Brewer:** None. **D. Li:** None. **D. Thal:** None. **A.C. Ludolph:** None. **K.M. Danzer:** None. **J.H. Weishaupt:** None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.03/O27

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: ALS Association

Title: Transplantation of human neural progenitor cells expressing GDNF into the motor cortex as a therapeutic strategy for treating amyotrophic lateral sclerosis

Authors: *G. M. THOMSEN, G. G. GOWING, J.-P. VIT, O. SHELEST, D. RUSHTON, P. SUEZAKI, C. N. SVENDSEN;
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Abstract: There is a lack of understanding in regards to the role that upper motor neurons play in amyotrophic lateral sclerosis (ALS) disease pathology. Our work builds on the hypothesis that upper motor neurons are a crucial component of ALS that should be therapeutically targeted. We have previously shown that the motor cortex is important in initiating disease onset in the SOD1G93A (SOD1) rat model of ALS by knocking down mutant SOD1 in the motor cortex alone. The mechanisms underlying this phenomenon however, remain unknown. It has been shown that glial cell line-derived neurotrophic factor (GDNF) can protect motor neuron function and survival in models of ALS. Here, human neural progenitor cells expressing GDNF (hNPC-GDNF) injected bilaterally and directly into the motor cortex of SOD1 rats survived and released GDNF, which was then taken up by surrounding neurons including corticospinal motor neurons. Critically, these targeted injections of hNPC-GDNF into only the motor cortex could significantly delay disease onset and extend survival. This current treatment strategy, unlike our previous study, does not involve altering mutant SOD1 expression, and is thereby more relevant to sporadic ALS cases. The novel approach of therapeutically targeting cortical motor neurons has great potential for a successful ALS treatment. Targeting the brain is much less invasive than targeting other affected central nervous system regions such as the spinal cord, making it ideal for fast translation to the clinic.

Disclosures: **G.M. Thomsen:** None. **G.G. Gowing:** None. **J. Vit:** None. **O. Shelest:** None. **D. Rushton:** None. **P. Suezaki:** None. **C.N. Svendsen:** None.

Poster

069. Motoneuron Disease

Location: Hall A

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH (R01 NS065808)

Legacy of Angels Foundation

Belgian American Educational Foundation

Chicago Biomedical Consortium

national Multiple Sclerosis Society

Title: The neurotoxin psychosine disorganizes plasma membrane lipids in the Krabbe disease

Authors: *L. D'AURIA[1], M. MARSHALL[1], E. LI[1], C. REITER[1], G. LI[2], R. VAN BREEMEN[2], M. ESCOLAR[3], E. BONGARZONE[1];
[1]Anat. and Cell Biol., [2]Medicinal Chem. and Pharmacognosy, UIC, Chicago, IL; [3]Children's Hosp., Univ. of Pittsburgs, Pittsburgs, PA

Abstract: Krabbe disease is a lysosomal storage disease with severe demyelination and neuronal dysfunctions. The disease is caused by a deficiency of galactosylceramidase and the toxic accumulation of the membrane-bound neurotoxin, psychosine, which activates intracellular pathogenic pathways by unknown mechanism(s). Because of its association with membranes, psychosine might initiate its toxicity by affecting the overall membrane and/or its sub-structures, the lipid domains. Indeed we have discovered that psychosine can accumulate in lipid rafts from Twitcher mice, a natural murine model for the disease. The goal of this study was to test the hypothesis that psychosine interferes with membrane fluidity and/or disrupts the architecture of specific plasma membrane lipid domains and can relay the toxicity into intracellular pathogenic pathway. For this, we first used plasma membranes from erythrocytes, a well-established featureless membrane model to study changes in membrane architecture and dynamics. Our work used, among others, cutting-edge techniques of high-resolution confocal microscopy, membrane fluidity measurement by LAURDAN-based dual-photon microscopy, fluorescence

recovery after photobleaching and fluorescence resonance energy transfer. Psychosine perturbs architecture of endogenous lipids. Concomitantly, the neurotoxic lipid induces focal changes of the overall rigidity of cell membranes as revealed by LAURDAN polarization. Psychosine also affects lipid dynamics since it specifically modulates the mobility of lipids. Equivalent experiments using oligodendrocytes (the main cell synthesizing psychosine in the brain) showed membrane reorganization upon exposure to psychosine which confirms its role in membrane perturbation. Altogether, these results are, for the first time, direct and quantitative evidence that psychosine's toxicity is initiated by perturbation of plasma membrane lipid domains. This work is relevant to understanding how psychosine causes toxicity in general and promotes myelin instability leading to demyelination in particular. These results may provide breakthroughs leading to alternative/complementary strategies to develop new therapies to cure the Krabbe disease. The fundamental discovery on how the neurotoxic psychosine affects the membrane can also provide mechanistic understanding of other sphingolipid storage diseases and demyelinating conditions such as Multiple Sclerosis.

Disclosures: **L. D'Auria:** None. **M. Marshall:** None. **E. Li:** None. **C. Reiter:** None. **G. Li:** None. **R. van Breemen:** None. **M. Escolar:** None. **E. Bongarzone:** None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.05/O29

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NRSA

MDA

CureSMA

Title: SMN functions as a chaperone for neuronal mRNP complex assembly

Authors: *P. G. DONLIN-ASP, C. FALLINI, J. P. ROUANET, S. P. H. HUANG, K. R. WILLIAMS, G. J. BASSELL, W. ROSSOLL;
Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Spinal muscular atrophy (SMA) is a neuromuscular disease characterized by a specific degeneration of motor neurons. SMA is caused by mutations leading to reduced levels of survival of motor neuron (SMN) protein, which is ubiquitously expressed with a well

characterized essential role in the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs). While general defects in splicing have been observed in SMA models, they are not unique to motor neurons. We have discovered specific defects in the axonal localization of mRNAs (β-actin, Gap43) and mRNA-binding proteins (HuD, IMP1) in axons and growth cones of SMN-deficient motor neurons. We also observed that overexpression of both HuD and IMP1 can restore axon outgrowth and GAP43 protein levels in growth cones of SMA motor neurons. Our findings led to the hypothesis that SMN plays a critical role in the assembly and/or trafficking of messenger ribonucleoproteins (mRNPs) in neuronal processes. To test our hypothesis, we have established a trimolecular fluorescence complementation (TriFC) assay in motor neuron cultures as a sensor for mRNA and protein association. Our findings reveal a deficiency in the assembly of IMP1 protein/β-actin mRNA containing complexes in SMA motor neurons. These results are further supported by immunopreciptation experiments from SMA tissue extracts, which show a substantial reduction in the levels of mRNAs associated with IMP1. In addition, size fractionation of neuronal RNA granules using density gradient centrifugation demonstrate a shift of mRNPs toward lighter fractions upon SMN depletion. We further show that SMN-deficiency results in defects in axonal local translation, likely as a consequence of impaired mRNP transport complex assembly. Local translation of β-actin and GAP43 are known to play an important role in axon outgrowth and branching. Taken together our findings reveal a novel function for SMN in the assembly of axonally transported mRNP complexes, and uncover effects on axonal mRNA localization and translation that may contribute to SMA pathology.

Disclosures: **P.G. Donlin-Asp:** None. **C. Fallini:** None. **J.P. Rouanet:** None. **S.P.H. Huang:** None. **K.R. Williams:** None. **G.J. Bassell:** None. **W. Rossoll:** None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.06/O30

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Target ALS

NIH Grant 1R01NS077863

Title: Vulnerable motor units become hypoexcitable before denervation in mouse models of ALS

Authors: L. MARTINEZ-SILVA, D. ZYTNICKI, *M. MANUEL;
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Abstract: One of the proposed mechanism to explain the death of motoneurons (MNs) in ALS is an excitotoxic process, i.e. an excess electrical activity leading to an overload of intracellular calcium triggering cell death. We have recently shown (Delestrée et al., J Physiol 2014) that spinal MNs of adult mice are not intrinsically hyperexcitable in SOD1-G93A mice. Instead, we found that a subpopulation of MNs lost their ability to fire repetitively in response to a stationary input. Furthermore, we have developed an *in vivo* adult mouse preparation allowing simultaneously recording intracellularly spinal MNs and recording the force developed by their motor unit (MU). Using this preparation we were able to identify the physiological type of recorded motor units. We were able to demonstrate that FF and FR MUs (that are vulnerable in ALS), but not the S type MUs (that are resistant in ALS), become progressively hypoexcitable before they lose their connections to their muscle fibers (Manuel et al., SfN 2014). To determine whether this phenotype is specific to SOD1-G93A mice or is a hallmark of the physiopathology of ALS, we performed experiments using a different mouse model, the FUS-P525L mouse (Sharma et al., submitted). The FUS gene has a very different cellular function than SOD1, and this model can thereby reveal alterations that are shared between different types of ALS. In this model, denervation occurs early (P30) in Tibialis Anterior (TA) and later (P180) in the Soleus (Sol) muscle, but it remains to be determined if this difference in the timing of denervation is due to muscle fiber composition or muscle function. We recorded motor units from ankle extensor muscles (including Sol), and ankle flexor muscles (including TA) at two time points (P30 and P180). Similarly to our previous report in SOD1 mice, preliminary experiments reveal that the excitability of motoneurons from ankle flexors at P180 is not different between mutant and control mice. For example, input conductance is 0.36 ± 0.12 uS (N=10) in mutants vs. controls (0.32 ± 0.12 uS; N=7), and rheobase is 8 ± 7 nA (N=7) vs. 6 ± 4 nA (N=5). However, a larger proportion of motoneurons are incapable of firing repetitively in response to stationary inputs in mutants (4/12) compared to controls (1/6). Remarkably, this deficit in excitability tends to affect larger FF and FR MUs. The excitability of S-type motoneurons remain to be studied, as muscles from ankle flexor muscles are mostly devoid of slow-contracting muscle fibers.

Disclosures: L. Martinez-Silva: None. **D. Zytnicki:** None. **M. Manuel:** None.

Poster

069. Motoneuron Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH/NINDS R01 NS088645

MDA 294842

Title: Neurodegeneration-linked nuclear loss of RNA/DNA binding protein TDP-43 impairs DNA double-strand break repair in neuronal genomes

Authors: *E. N. GUERRERO[1,2,3], J. MITRA[2], P. HEGDE[2], H. WANG[2], J. RAO[1], M. HEGDE[2];
[1]INDICASAT-AIP, Panama, Panama; [2]Radiation Oncology, Houston Methodist Res. Inst., Houston, TX; [3]Biotech., Acharaya Nagarjuna Univ., Guntur, India

Abstract: TAR DNA-binding protein 43 (TDP-43),is an RNA/DNA-binding protein involved primarily in RNA processing, but its cytoplasmic aggregates have been found to be a pathological hallmark in motor neurons affected with Amyotrophic Lateral Sclerosis (ALS). In normal neurons, TDP-43 shuttles between nucleus and cytoplasm due to its function in RNA processing; but TDP-43 also binds to DNA, although its DNA-binding functions have not been explored. A significant accumulation of genomic damage is observed in TDP-43-linked diseases and previous studies identified a DNA repair protein "Ku" in a TDP-43 immunocomplex from human cells. In this study we investigated the possibility of TDP-43's involvement in DNA damage repair; a previously unexplored area. We used *in situ* Proximity Ligation Assay (PLA) to examine in cell interaction of TDP-43 with double strand breaks repair (DSBR) proteins in human neural stem cell (hNSC) line differentiated into spinal motor neurons. The PLA showed a strong interaction between TDP-43 with DSBR proteins Ku70, DNA-PKcs, XRCC4 and DNA ligase IV. The TDP-43's association with DSBR proteins was significantly enhanced in cells exposed to DSB-inducing ionizing radiation (IR). The in cell association was confirmed by immuno-precipitation (IP) analysis with endogenous TDP-43 and FLAG-TDP-43 ectopically expressing cells, including enhancement of interaction after IR. Neutral and Alkaline comet assay analysis in TDP-43 depleted cells showed a persistent accumulation of DSBs in the absence of any genotoxic agent which is highly significant. Loss of TDP-43 affected recruitment of 53BP1 and XRCC4/DNA LigaseIV complex (two key components of non-homologous end joining mediated DSB repair pathway) at DSB sites. Together these data strongly suggest a role of TDP-43 in efficient DSB repair in neuronal genomes and its pathological linkage to neurodegeneration.

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Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.08/O32

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: ANR

IRME

Title: Subclinical abnormal sensory-motor integrations at spinal level in ALS patients

Authors: *V. MARCHAND-PAUVERT;
U1146, Inserm, Paris, France

Abstract: Using electrophysiology and spinal diffusion tensor imaging, we recently confirmed early sensory defects in patients with amyotrophic lateral sclerosis (ALS) who did not exhibit any sensory impairment when assessed using routinely used clinical scales(1). We have stimulated the median and ulnar nerves at writ level to investigate the sensory evoked potentials (SEPs) produced by afferent inputs from the hand area. In parallel, we have tested the sensory-motor integrations at motoneuron level. For this, we combined transcranial magnetic stimulation (TMS) over the primary motor cortex, with median and ulnar nerve stimulations. All the patients exhibited hand motor weakness but no proximal motor defects, especially in triceps brachii. We first investigated the recruitment curve of motor evoked potentials (MEP) in triceps brachii to investigate the corticospinal excitability. Then, TMS (at about I50) was combined to median or ulnar nerve whose intensity was adjusted at 6 x the perceptual threshold in order to activate group Ia afferent fibers from intrinsic hand muscles which are known to have monosynaptic projections onto triceps motoneurons(2). We compared the size of the MEP produced by isolated TMS and by combined stimulation. Except an increase in MEP threshold in ALS patients (n=21), the MEP recruitment curves were similar to those in gender and age matched controls (n=21). Despite a similar mean size of MEP produced by isolated TMS between groups, the MEPs produced on combined stimuli were significantly larger in ALS compared to controls. In the same group of participants, the peripheral SEPs were smaller in ALS(1). Therefore, despite less sensory afferent inputs at spinal level and similar corticospinal excitation, we observed an enhanced excitation at motoneuron level in ALS. Given the absence of evidences for excitability changes at motoneuron level, the results suggest specific changes at the level of the synapses between group Ia afferents and motoneurons leading to hyperexcitability. One possible mechanism would be a change in the control of primary afferent depolarization interneurons mediating presynaptic inhibition of group Ia afferents to compensate the sensory de-afferentation. This enhanced synaptic activity may contribute to the motoneuron excito-toxicity, which is among the causes of motor neuron death in ALS. Given the diffuse monosynaptic

excitations from Ia inputs to human upper limb motoneurons(3), especially from distal muscles to more proximal muscles, such a mechanism would participate in the propagation of motor neuron disease. (1) Iglesias et al. BMJ Open 2015. (2) Lourenço et al. EBR 2007. (3) Marchand-Pauvert et al. J Phys 2000.

Disclosures: V. Marchand-Pauvert: None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant RO1

Title: Non-invasive muscle impedance measurements correlate to muscle force in ALS mice

Authors: *J. LI, B. SANCHEZ, A. PACHECK, S. RUTKOVE;
Neurol., Harvard Med. School, Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: Objective: Evaluate the relationship between force generating capability and muscle impedance in ALS mice. Methods: Twenty eight ALS (B6SJL-Tg(SOD1-G93A)1Gur/J) mice, between 13 and 18 weeks of age, obtained from Jackson Laboratories were examined. *In vivo* muscle electrical impedance myography (EIM), *in situ* isometric force, and the compound motor action potential (CMAP) were measured from a unilateral gastrocnemius. . Body weight, paw grip strength, and muscle weight were also measured. Results: Higher resistance, lower phase but not much differences of reactance were found in mice with more advanced ALS. For instance, at 50 kHz, 18-week old animals had on average a 33% higher resistance than 13-week old animals (375 ± 17.2 Ω vs. 283 ± 6.68 Ω respectively, $p < 0.01$); and on average a 23% smaller phase than 13-week old animals (13.7 ± 0.82 ° vs. 17.8 ± 1.33 ° respectively, p 0.05). Additionally, a modeled multifrequency impedance parameter, the center frequency, was also evaluated. It was markedly higher (93% on average) in the 18-week (53.5 ± 5.43 kHz) versus 13-week (103 ± 16.9 kHz) old mice ($p = 0.05$). Similarly, the 18-week old mice showed weaker grip strength than 13-week old mice. For the front paw, there was a significant difference (63% on average, 0.03 ± 0.01 kg vs. 0.08 ± 0.01 kg respectively, p 0.05). Force generating capability was evaluated by both maximum force and maximum velocity under tetanic stimulation. Maximum force was 17% lower on average for 18-week (934 ± 116 mN) than for the 13-week old mice (1119 ± 168 mN); Maximum velocity was 14% lower on average for 18-week (33.6 ± 1.21 mm/s) than 13-week old

mice (39.1 ± 2.33 mm/s). Nevertheless, none of their differences were statistically significant. There were strong correlations among EIM parameters, front paw grip strength, and force. For example, phase correlates to front paw grip strength with r as 0.74 ($p < 0.001$); to maximum force with r as 0.48 ($p < 0.02$). Conclusions: Impedance measures are sensitive to the progressive deterioration in strength in ALS mice, with major alterations in the 50 kHz phase and the resistance parameters, and very large increase in the center frequency parameter in the oldest mice. These alterations correspond closely with the observed alterations in force production. The results support that non-invasive impedance measurements could serve as a useful easily obtained surrogate measure of weakness in ALS.

Disclosures: **J. Li:** None. **B. Sanchez:** None. **A. Pacheck:** None. **S. Rutkove:** F. Consulting Fees (e.g., advisory boards); Skulpt Inc.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.10/O34

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: ANR

IRME

Title: Early and late somatosensory evoked potential impairment in amyotrophic lateral sclerosis

Authors: *S. SANGARI[1], V. MARCHAND-PAUVERT[2];
[1]UMPC/INSERM/CNRS, Paris, France; [2]U1146, INSERM, Paris, France

Abstract: Amyotrophic lateral sclerosis (ALS) is an adulthood neurodegenerative disease characterized by the loss of cortical and spinal motor neurons, leading progressively to muscle weakness, paralysis and death. Often considered as a purely motor disease, evidences of sensory pathway involvement are emerging. Study of Somatosensory Evoked Potential (SEP) allows subclinical assessment of proprioceptive pathways at peripheral (Erb point) and cortical (parietal cortex) level. Several SEP studies have been carried out on ALS patients: evidences have been brought for amplitude reduction with central but no delayed peripheral latency, and SEP from lower limbs are more altered than those from upper limbs while no patients exhibited clinical sensory troubles. To our knowledge, SEP studies focused early SEP (N9 and 20) but none on late SEP (latency > 35 ms). It is admitted that early SEP are generated from thalamo-cortical

projections onto primary somatosensory area (S1) and its relationships with the motor and pre-motor areas. The late SEP are generated from secondary somatosensory area (S2). In our previous study (Iglesias & Sangari et al. 2015), we have shown that the amplitudes of N9 and N20 produced by median and ulnar nerve stimulation at wrist level and the central conduction velocity were reduced in ALS patients compared to gender and age matched controls. Moreover, we observed a reduction in the following early (P25-N35) and late SEP recorded over the contra- and the ipsi-parietal cortex. This raises the questions whether i) the reduction of early and late SEP in ALS patients would be correlated to N9 reduction (and thus due to less sensory inputs), or would include excitability changes of pons-thalamus relay and/or cortico-sub-cortical interaction, and ii) the reduction of SEP recorded ipsi-laterally would be just far-field potentials from contralateral cortex or due to transcallosal or ipsi-parietal cortex impairment. We performed electroencephalographic recording on 15 healthy participants at the Erb point, bilateral parietal and occipital cortices. We electrically stimulated median and ulnar nerve at wrist level at 1.5, 3, 6 and 9 time the perceptual threshold (PT). Preliminary results showed independent relation between stimulation intensity and potentials: N9 increased linearly with stimulus intensity, early SEP reached a plateau at 6xPT, and late SEP reached quickly a saturation plateau at 3xPT and then decreased. Ipsilateral potentials recorded seem to be far-field potentials from de contra-parietal side. Thus, it seems that ALS patients exhibit relay and cortico-sub-cortical impairment of the contra-somatosensory area, early in the disease.

Disclosures: **S. Sangari:** None. **V. Marchand-Pauvert:** None.

Poster

069. Motoneuron Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R01 NS085207

ALSA

MDA

Robert Packard Center

Jean and Pape Adams Foundation

William and Ella Owens Foundation

NIH NIA

Title: Role of ADARB2 in GLUA2 editing deficiency in C9ORF72 amyotrophic lateral sclerosis and frontotemporal dementia

Authors: *E. F. MENDEZ[1], E. L. DALEY[2], X. TANG[2], S. VIDENSKY[2], R. SATTLER[2]; [2]Neurol., [1]Johns Hopkins Univ., Baltimore, MD

Abstract: A mutation consisting of a GGGGCC (G4C2) hexanucleotide repeat expansion (HRE) sequence in the chromosome 9 open reading frame 72 (C9orf72) gene has been identified as the most common cause of both familial and sporadic amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). This mutation, when transcribed, causes RNA to form toxic foci that sequester RNA binding proteins, including a member of the RNA-specific adenosine deaminase (ADAR) family, ADARB2. Using patient-derived iPS neurons and postmortem patient tissue, we show that the (G4C2) mutation causes RNA editing deficits of the GluA2 α-amino-3-hydroxyl-5-methyl-4-isoxazole propionate AMPA receptor subunit and we implicate that a combination of ADARB2 sequestration and reduced ADARB1 transcript levels are involved in this phenomenon. This misediting is suggested to lead to increased susceptibility to glutamate-induced excitotoxicity observed in C9orf72 mutant iPS neurons - a hallmark mechanism known to contribute to motor neuron loss. In addition, lack of editing of GluA2 is likely to contribute to cognitive impairment and synaptic dysfunction as observed in both C9orf72 ALS and FTD patients.

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Poster

069. Motoneuron Disease

Location: Hall A

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Program#/Poster#: 69.12/O36

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Helmholtz Virtual institute “RNA dysmetabolism in Amytrophic Lateral Sclerosis and Frontotemporal Dementia”

Title: PGC-1alpha signalling system in mouse models of FUS- and SOD1-related amyotrophic lateral sclerosis

Authors: H. BAYER[1], K. LANG[1], J. HANSELMANN[1], I. MERDIAN[1], L. DUPUIS[2], *P. WEYDT[1], A. WITTING[1];
[1]Dept. of Neurol., Ulm Univ., Ulm, Germany; [2]INSERM, Strasbourg, France

Abstract: Amyotrophic lateral sclerosis (ALS) is characterized by hypermetabolism. Genetic variants of a brain-specific promoter of the metabolic transcriptional coactivator peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1-α (PGC-1α) modify age of onset in human ALS1 suggesting that changed function or activity of PGC-1α contributes to the metabolic changes in ALS. We investigated the PGC-1α expression and function in different tissues and cells of two ALS mouse models, SOD1(G93A) mice and a knock-in Fus mouse model deleted of the nuclear localization signal (FusΔNLS). We showed that the canonical and brain-specific PGC-1α isoforms are reduced in spinal cord and brain stem of diseased mice from the SOD1 mouse model, whereas PGC-1α remains unchanged in unaffected brain regions and in non-diseased FusΔNLS/+ mice. In contrast, in peripheral tissues like brown adipose tissue and muscle PGC-1α was upregulated in SOD1 mice during disease progression. In primary neurons and glia cells of both mouse models the basal expression of PGC-1α was unchanged in comparison to corresponding controls. In contrast, in primary brown adipocytes of FusΔNLS/ΔNLS mice basal levels of Pgc-1α and Ucp-1 were increased. To investigate the effect of ALS-associated mutations we stimulated the PGC-1α signaling pathway by lactate in neurons and norepinephrine in adipocytes. The lactate-induced PGC-1α expression and signaling was not influenced by the tested ALS-associated mutations. In contrast, in primary brown adipocytes of FusΔNLS/ΔNLS mice stimulation with norephinephrine resulted in a stronger increase of Pgc-1α and Ucp-1 levels. So far, our results suggest that PGC-1α expression is changed in the CNS and peripheral tissues in diseased animals. In contrast to SOD1 mutations, ALS-associated Fus mutations increase the PGC-1α signaling in adipocytes which would contribute to the hypermetabolism of ALS.

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Poster

069. Motoneuron Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS061696

NIH Grant NS081426

Title: Leveraging informatics to assess the SOD1 G93A amyotrophic lateral sclerosis mouse model

Authors: *R. KIM, C. IRVIN, J. KNIPE, C. S. MITCHELL;
Georgia Inst. of Technol., Atlanta, GA

Abstract: The superoxide dismutase-1 glycine 93 to alanine (SOD1 G93A) transgenic mouse model has been a mainstay in the field of Amyotrophic Lateral Sclerosis (ALS) for over two decades with hundreds of published studies depicting relationships and observations thought to contribute to the disease etiology. It has been our ongoing goal to leverage this extensive research utilizing informatics-based approaches. To this end, we continue to build a comprehensive database that includes figure captions and quantitative data recaptured from over 2,000 published articles. A searchable SOD1 G93A figure caption database is freely available on our website, www.pathology-dynamics.org. Using our comprehensive database, we present an informatics-based systematic review of the field's primary experimental research to determine the distribution of article belonging to the following ontological categories: axonal transport, apoptosis, cellular chemistry, excitability, energetics, oxidative stress, inflammation, proteomics and protein aggregation, and systemic contributors and effects. Additionally, we present example informatics-based meta-analyses of published temporal experimental data examining mitochondrial function, calcium, and oxidative regulation. Finally, we present preliminary results of a multi-treatment meta-analysis to predict which ontological category(s) of treatments appear the most promising for future preclinical study.

Disclosures: **R. Kim:** None. **C. Irvin:** None. **J. Knipe:** None. **C.S. Mitchell:** None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.14/O38

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: HHMI

Biogen, IDEC

Title: Analysis of SOD1 transmission to motor neurons in a SOD1G85R mouse amyotrophic lateral sclerosis (ALS) model

Authors: *E. V. THOMAS[1], W. A. FENTON[2], M. NAGY[1], D. LI[1], J. M. MCGRATH[3], A. L. HORWICH[4];
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Abstract: Transmission of aggregation-prone proteins, such as tau, alpha-synuclein, amyloid beta, and mutant SOD1, from one neuron to another has been hypothesized to contribute to neurodegeneration in patients and mouse models. In fact, anatomical progression of disease symptoms in amyotrophic lateral sclerosis (ALS) patients suggests spread of pathology in the spinal cord. Mutations in superoxide dismutase 1 (SOD1) cause approximately 20% of familial ALS cases. SOD1 mutations cause protein aggregation in motor neurons in the spinal cord; therefore, motor neuron demise in ALS patients is thought to be due to a toxic gain-of-function as a result of SOD1 misfolding. SOD1 aggregates can be taken up by dividing cells in culture, and injection of spinal cord lysate from diseased SOD1G93A transgenic mice can accelerate ALS pathogenesis in a low-copy SOD1G85R-YFP transgenic mouse strain. These data ask whether mutant SOD1 can be taken up by motor neurons in the spinal cord *in vivo*, thereby causing disease spread. We have modeled SOD1-linked ALS using transgenic mice expressing G85R SOD1-YFP. G85R SOD1-YFP mice develop large, cytosolic, YFP-fluorescent aggregates in motor neurons and paralyze by six months of age, while wt SOD1-YFP mice do not develop aggregation or motor neuron disease symptoms. In order to visualize SOD1 propagation in motor neurons and assess its contribution to ALS pathogenesis, we generated a second transgenic mouse strain expressing G85R SOD1-CFP. Chimeric mice were then formed from embryos of the two G85R SOD1-FP strains resulting in spinal cord neurons expressing one or the other fluorescently labeled mutant SOD1. Transfer of mutant SOD1 between neurons will result in cells co-labeled by both the endogenously expressed mutant SOD1 and SOD1 derived from cells expressing the other fluorescently-tagged mutant. Our studies indicate that while no mutant SOD1 is transferred at early time points (1 month), small amounts of SOD1 can be transmitted preferentially to motor neurons at later time points (3 months) in symptomatic, SOD1 chimeric mice. Of note, other neuronal sub-types do not exhibit CFP/YFP co-labeling at three months. These results are a proof-of-principle that a predominantly cytosolic protein can exit cells and be taken up by motor neurons *in vivo*, a necessary step if misfolded SOD1 is to template aggregation in recipient cells. However, the exact contribution of SOD1 intercellular propagation to disease is unclear. These data support the need for further studies to address the mechanism and pathogenicity of SOD1 protein transfer, as interruption of this process may comprise a therapeutic avenue in ALS patients.

Disclosures: E.V. Thomas: None. **W.A. Fenton:** None. **M. Nagy:** None. **D. Li:** None. **J.M. McGrath:** None. **A.L. Horwich:** None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant R01-NS044292

Title: Effects of caspase-3 cleavage-resistant Eaat2 on the Sod1-G93A mouse model of amyotrophic lateral sclerosis

Authors: *L. T. ROSENBLUM[1], P. PASINELLI[2], D. TROTTI[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a rapidly progressive and fatal neurodegenerative disease that primarily affects the corticospinal tract. In ALS, astrocytes cause toxicity to upper and lower motor neurons in a process known as non-cell autonomous toxicity. It has previously been shown that in spinal cord astrocytes of the SOD1-G93A ALS mouse model, activated caspase-3 cleaves the glutamate transporter, EAAT2, leading to the accumulation of a sumoylated C-terminal fragment (CTE-SUMO1) as disease progresses. Furthermore, expression of an artificial CTE-SUMO1 construct by astrocytes, mimicking the *in vivo* astrocytic accumulation, causes the secretion of one or more factors that are toxic to motor neurons in *in vitro* experiments. We sought to determine the significance of this pathway *in vivo* by genetically blocking the caspase-3 cleavage of EAAT2 in a novel mouse model. We report here the generation of a knock-in mouse model with a point mutation on the astrocytic glutamate transporter, EAAT2, which prevents cleavage by activated caspase-3 (EAAT2-D504N). EAAT2-mutant mice were crossed with the SOD1-G93A mouse model of ALS to generate SOD1-G93A mice homozygous for either EAAT2-WT or EAAT2-D505N. Non-SOD1-G93A mice homozygous for EAAT2-WT or EAAT2-D505N were also generated and used as healthy controls. Mice were followed past post-natal day 160, the normal end-stage for SOD1-G93A mice, and assessed for disease progression by pathology and behavioral assessments.

Disclosures: L.T. Rosenblum: None. **P. Pasinelli:** None. **D. Trotti:** None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.16/O40

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: 2013 MSCRFF-034

Title: Astrocyte Cx43 contributes to motor neuron toxicity in ALS

Authors: *A. A. ALMAD[1], A. DORESWAMY[2], S. K. GROSS[2], N. HAUGHEY[3], N. J. MARAGAKIS[2];
[2]Neurol., [3]Neuropathology, [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease resulting in progressive degeneration of motor neurons (MN) in the brain and spinal cord leading to weakness and death. Astrocytes play a critical role ALS influencing course of disease and contributing to MN death. Studies from *in vitro* and *in vivo* models demonstrate astrocytes are altered in ALS and in turn affect MN survival. Here we investigate a potential mechanism through which astrocytes lead to MN toxicity. Astrocytes are interconnected through connexins (Cx) and Cx43 is a major astrocyte connexin conducting crucial homeostatic functions in the CNS. Under pathological conditions, connexins are altered and their functions are compromised as observed in spinal cord injury, Alzheimer's disease, Parkinson's, ischemia, and others. We hypothesized that abnormal Cx expression serves as a potential mechanism for astrocyte-mediated toxicity in ALS. We examined if Cx43 is altered during course of the disease using $SOD1^{G93A}$ mouse model of ALS and observed Cx43 protein increased at disease onset and remained significantly elevated in the spinal cord of endstage mice. Notably, this increase in Cx43was observed not only in ALS mouse model but also in motor cortex and spinal cord of ALS post-mortem patients compared to age-matched controls. To investigate if these changes in Cx are specific to astrocytes, we isolated astrocytes from $SOD1^{G93A}$ and $SOD1^{WT}$ mice and characterized their Cx43 expression and function. We found even in the absence of neurons, Cx43 expression was elevated in $SOD1^{G93A}$ astrocytes compared to control. This increase in Cx43 protein expression translated to alteration in functional properties of mutant astrocytes as we conducted Cx specific assays and found that mutant astrocytes exhibited enhanced dye spreading, hemichannel mediated uptake and intracellular calcium levels compared to control astrocytes. Finally, to understand the impact of increased expression of Cx43 on survival of MNs, we conducted co-culture experiments between astrocytes and HB9-GFP positive MNs. We tested if blocking Cx43 protects MNs from the toxic effects of $SOD1^{G93A}$ astrocytes and monitored their survival in the presence or absence of Cx43 mimetic peptide. We noted significantly better survival of MNs on top of $SOD1^{G93A}$ astrocytes treated with Cx43 peptide blocker versus without the peptide. These results demonstrate aberrant Cx properties of ALS astrocytes and indicate blocking Cx43 confers neuroprotection to MNs. These novel findings have widespread implications for other neurodegenerative diseases as well and provides a therapeutic strategy targeting glial cells to protect the vulnerable MNs.

Disclosures: **A.A. Almad:** None. **A. Doreswamy:** None. **S.K. Gross:** None. **N. Haughey:** None. **N.J. Maragakis:** None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.17/O41

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: BMBF GO-Bio Grant FKZ: 031A386

Title: Disease-promoting immunological alterations in patients with amyotrophic lateral sclerosis

Authors: *S. T. PETERS[1], S. KÜSPERT[1], E. ZITZELSPERGER[1], S. KLATT[2], M. RIEMENSCHNEIDER[3], L. AIGNER[4], T.-H. BRUUN[1], U. BOGDAHN[1];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) also known as Lou Gehrig´s disease, represents a progressive and fatal neurodegenerative disorder affecting motor neurons with a typical median disease course of 2 to 5 years and a lifetime risk of 1:400 (Ingre et al. 2015). Despite the expanding research interest, the heterogenous etiology of ALS in combination with the lack of validated biomarkers and active therapeutic agents obstruct effective treatment of this orphan disease. In the last decades a growing number of studies including cell culture experiments, animal models, but also human studies indicate immune and inflammatory abnormalities to contribute to the pathogenesis of ALS (McCombe et al. 2011). It has been demonstrated that during disease progression a protective Type 2 (pre-symptomatic or stable disease) traverses into a neurotoxic Type 1 immune response (progressive disease) (Murdock et al. 2015). Here we first reveal the activation state of the TGF-β pathway incl. most important downstream molecules within spinal cord (SC) and motor cortex (MC) tissue homogenates (kindly provided by Prof. Thal, MND network Germany) of ALS and control patients via Western Blot analysis and *in situ* hybridisation. Next, we investigated the neurogenesis in SC and MC tissue of ALS and controls by measuring the expression profiles of early neuronal differentiation and proliferation markers. Subsequently, we examined the SC and MC tissue for a correlation of tissue-infiltrating immune cells and local neuronal, glial, and microglial components. Finally, we determined the hematological alterations of plasma samples obtained from ALS patients and healthy controls regarding their levels of leukocytes, chemokines, pro- and anti-inflammatory cytokines, and

vascular as well as angiogenic factors using electrochemoluminescence and FACS analysis. We were able to demonstrate enhanced circulating levels of pro-inflammatory cytokines (IFNγ, TNFα, IP-10) within the serum of ALS patients compared to healthy controls. Further, a trend towards reduced expression of Tie-2 and VEGF-C indicated diminished angiogenesis in ALS patients. Finally, there was a positive correlation between the circulating levels of monocytes and the ALS-FRS-R and an inverse correlation of mobilized eosonophils and the ALS-FRS-R with the latter being confirmed by enhanced levels of plasma eotaxin of ALS patients compared to healthy controls. Taken together, the results of the current study might shed some light on biomarker discovery and provide possible purchases for a successful treatment development in ALS.

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Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.18/O42

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Antisense oligonucleotide treatment protects against neuromuscular denervation in the SOD1-G93A mouse model of ALS as evaluated by a pre-symptomatic electrophysiological measure

Authors: *B. J. FARLEY[1], N. T. COMFORT[1], J. L. GOODMAN[1], A. M. KUSZPIT[1], T. COLE[2], H. KORDASIEWICZ[2], E. SWAYZE[2], A. MCCAMPBELL[1], M. WITTMANN[1]; [1]Biogen, Cambridge, MA; [2]Isis Pharmaceuticals, Carlsbad, CA

Abstract: ALS is a neurodegenerative disease characterized by progressive motor neuron loss and eventual death. Mice overexpressing a mutant form of human SOD1, a gene linked to approximately 20% of familial ALS cases, represent a widely used ALS model. These mice recapitulate many of the histological and behavioral characteristics of the human disease. We evaluated whether an antisense oligonucleotide (ASO) directed against the mutant SOD1 expressed in SOD1-G93A mice (B6.Cg.-Tg(SOD1*G93A)1Gur/J) could rescue deficits present in an electrophysiological measure, compound muscle action potential (CMAP), of neuromuscular denervation. We first characterized the timecourse of CMAP deficits in SOD1-G93A mice. CMAP recordings were performed under anesthesia every two weeks from week 5 to week 13, with CMAP amplitudes being measured from the tibialis anterior muscle in response

to sciatic nerve stimulation. Deficits in CMAP amplitude were present in untreated SOD1-G93A mice relative to WT mice (two-way ANOVA, $p<0.0001$) beginning at week 7 and persisted thereafter. Thus, the electrophysiological deficits are present at least 10 weeks before symptom onset is known to occur, confirming CMAP in tibialis as an early and sensitive pre-symptomatic measure for evaluating disease course in this model. In a second experiment, mice were treated with an ASO targeted against mutant SOD1 (SOD1-ASO) at week 5.5. We found that intra-cerebroventricular injections of SOD1-ASO protected against the CMAP decline observed in sham-treated SOD1-G93A mice (two-way ANOVA, $p<0.0001$), with CMAP values being higher in treated than sham-treated mice beginning at week 7 and the effect persisting through week 13, the last time point measured. At this last time point, CMAP values were indistinguishable between WT and ASO-treated SOD1-G93A mice (WT: 74 ± 4 mV; SOD1 ASO-treated SOD1-G93A: 70 ± 2 mV; sham-treated SOD1-G93A: 31 ± 3 mV). These results demonstrate that a SOD1-ASO injected at an early time point protects against neuromuscular denervation in this model and that this treatment effect can be measured pre-symptomatically using electrophysiology.

Disclosures: **B.J. Farley:** A. Employment/Salary (full or part-time):; Biogen. **N.T. Comfort:** A. Employment/Salary (full or part-time):; Biogen. **J.L. Goodman:** A. Employment/Salary (full or part-time):; Biogen. **A.M. Kuszpit:** A. Employment/Salary (full or part-time):; Biogen. **T. Cole:** A. Employment/Salary (full or part-time):; Isis Pharmaceuticals. **H. Kordasiewicz:** A. Employment/Salary (full or part-time):; Isis Pharmaceuticals. **E. Swayze:** A. Employment/Salary (full or part-time):; Isis Pharmaceuticals. **A. McCampbell:** A. Employment/Salary (full or part-time):; Biogen. **M. Wittmann:** A. Employment/Salary (full or part-time):; Biogen.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.19/O43

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: GSK-BBSRC Industrial CASE studentship

Title: Inhibition of p38 MAPK alpha rescues retrograde axonal transport defects in ALS

Authors: ***K. L. GIBBS**[1], B. KALMAR[1], M. AHMED[2], E. BROWNE[2], C. DAVIES[2], L. GREENSMITH[1], G. SCHIAVO[1];
[1]Sobell Dept. of Motor Neurosci. and Movement Disorders, Inst. of Neurol., London, United

Kingdom; [2]GlaxoSmithKline Res. and Develop. China Singapore Res. Ctr., The Helios, Singapore

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by the degeneration of both upper and lower motor neurons. Defects in retrograde axonal transport have been found pre-symptomatically in the $SOD1^{G93A}$ mouse model of ALS. However, the exact role that these defects play in ALS disease pathogenesis is not yet fully understood and still remains a subject of much controversy in the field. In order to address this, we screened a library of small molecule inhibitors in search of pharmacological enhancers of retrograde axonal transport in motor neurons. Several inhibitors of p38 MAPK were identified in our screen and were subsequently shown to correct deficits in the retrograde axonal transport of signalling endosomes *in vitro* in primary $SOD1^{G93A}$ motor neurons. Knockdown of p38 MAPK isoforms using lentiviral vectors revealed that p38 MAPK alpha was responsible for the transport deficits observed in $SOD1^{G93A}$ motor neurons. Using *in vivo* imaging of the sciatic nerve in live anaesthetised mice, we found that a specific inhibitor of p38 MAPK alpha and beta could rescue axonal transport impairments in early symptomatic $SOD1^{G93A}$ mice. Our findings demonstrate for the first time the pathogenic effect of p38 MAPK alpha over-activation on retrograde axonal transport. Since axonal transport deficits have been implicated in the pathogenesis of ALS, the ability to pharmacologically reverse these defects may represent a novel therapeutic strategy for ALS.

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Poster

069. Motoneuron Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NINDS Grant NS07786303

T32 MRS Training Grant

Title: S6K-mediated cellular change in a mouse model of ALS

Authors: *S.-W. KUO[1], C. J. HECKMAN[1,2];
[1]Dept of Physiol., Northwestern Univ., Chicago, IL; [2]Rehabil. Inst. of Chicago, Chicago, IL

Abstract: In ALS, spinal motoneurons with various sizes show distinctive degenerating rates in which large motoneurons are particularly vulnerable. ALS spinal motoneuron size is increased since neonatal stage accompanied with abnormal electrical properties. Motoneuron size enlargement may be the subsequence of initial responses to morbific prodromal factors such as altered expression level of proteins related to survival and translation. We hypothesize that the early-stage abnormal soma size and electrical properties might link to the selective susceptibility of motoneurons in ALS. S6K is selected for motoneuron size manipulation due to its regulatory roles in protein translation, cell growth and synapse development. Our results showed that neonatal motoneuron size was abnormally enlarged, and that S6K inhibitor could maintain the size of lumbar spinal motoneurons in early-stage G93A mice. Post-symptomatic S6K inhibitor administration onto adult mice revealed distinctive responses upon different doses. Lower dose mildly prolonged the lifespan, while the higher does significantly accelerated the disease progression and shorten lifespan. The early-stage abnormality can be amended by S6K-suppression implies the involvement of S6K pathway in ALS pathogenesis. The distinctive dose-dependent responses at late-stage suggested the importance of S6K management in ALS.

Disclosures: S. Kuo: None. **C.J. Heckman:** None.

Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.21/O45

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Supported by MURST (PRIN project 2006058401)

Title: Exocytosis regulates the trafficking of GABA and glycine heterotransporters in spinal cord glutamatergic synapses: a mechanism for the excessive heterotransporter-induced glutamate release in amyotrophic lateral sclerosis

Authors: *C. USAI[1], M. MILANESE[2], T. BONIFACINO[2], E. FEDELE[2], C. REBOSIO[2], L. CATTANEO[2], F. BENFENATI[3], G. BONANNO[2];
[1]Natl. Rese Council, Genova, Italy; [2]Dept. of Pharmacy, Pharmacol. and Toxicol. Unit - Ctr. of Excellence for Biomed. Res., Univ. of Genoa, Genova, Italy; [3]Dept. of Neurosci. and Brain Technol. and Dept. of Exptl. Med., Italian Inst. of Technol. and Univ. of Genoa, Genova, Italy

Abstract: Protein trafficking is a crucial process in CNS plasticity. Several evidences show that functional membrane proteins can be rapidly trafficked from the cytosol to the plasma membrane

and vice-versa, exploiting endo/exocytotic events (Buckley et al., J Physiol. 2000, 525:11-19,). In particular it has been proposed that transporters for a given neurotransmitter are rapidly trafficked to and from the plasma membrane in relation to the exocytotic release of the neurotransmitter it self and that this type of trafficking is important in regulating neuronal signaling (Geerlings et al., J BiolChem., 2001, 276:17584-90; Deken et al., J Neurosci. 2003, 23:1563-68). The impact of synaptic vesicle endo-exocytosis on the trafficking of nerve terminal heterotransporters was studied by monitoring membrane expression and function of the GABA transporter-1 (GAT-1) and of type-1/2 glycine (Gly) transporters (GlyT-1/2) at spinal cord glutamatergic synapses. Experiments were performed by inducing exocytosis in wild-type (WT) mice, in amphiphyisin-I knockout (Amph-I KO) mice, which show impaired endocytosis, or in SOD1G93A mice as a model of human amyotrophic lateral sclerosis that shows a constitutively excessive Glu exocytosis (Milanese et al., J Neurochem. 2011, 116:1028-42). Exposure of spinal cord synaptosomes from WT mice to a 35 mM KCl pulse increased the expression of GAT-1 and GlyT-1/2 at glutamatergic synaptosomal membranes and enhanced the GABA and glycine-induced glutamate (Glu) release. Preventing depolarization-induced exocytosis normalized the excessive GAT-1 and GlyT-1/2 heterotransporter-induced Glu release in WT mice. Impaired endocytosis in Amph-I KO mice increased GAT-1membrane expression, the GABA uptake and the GAT-1 heterotransporter-evoked release of Glu in spinal cord synaptosomes. The constitutively excessive Glu exocytosis in SOD1G93A mice resulted in augmented GAT-1 and GlyT-1/2 expression at glutamatergic synaptosomal membranes paralleled by an increased of GABA and glycine-induced Glu release. Thus, endo-exocytosis regulates the trafficking of GAT-1 and GlyT-1/2 heterotransporters sited at spinal cord glutamatergic nerve terminals. As a consequence, it can be hypothesized that the excessive GAT-1 and GlyT-1/2 heterotransporter-mediated Glu release (Raiteri et al., 2005, Neurotoxicol. 26:883-92; Milanese et al., 2010, J Neurochem. 113:489-501), in the spinal cord of SOD1G93A mice, is due to the heterotransporter over-expression at the nerve terminal membrane, promoted by the excessive Glu exocytosis.

Disclosures: **C. Usai:** None. **M. Milanese:** None. **T. Bonifacino:** None. **E. Fedele:** None. **C. Rebosio:** None. **L. Cattaneo:** None. **F. Benfenati:** None. **G. Bonanno:** None.

Poster

069. Motoneuron Disease

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Program#/Poster#: 69.22/O46

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS069616

NIH Grant NS081426

Title: A temporal assessment of excitability in the SOD1 G93A Amyotrophic Lateral Sclerosis mouse model

Authors: *I. VERNON, Y. SHI, M. JENSEN, Z. SEILING, C. S. MITCHELL;
Georgia Inst. of Technol., Atlanta, GA

Abstract: Excitotoxicity occurs when an abnormal amount of glutamate is present in the synaptic cleft of the pre-and post- synaptic neuron, which activates both AMPA and NMDA ionotropic receptors, causing the postsynaptic neuron to die of toxic overstimulation. Abnormal amounts of glutamate could be due to the presynaptic neuron over-releasing the neurotransmitter or due to the inability of the glutamate transporters, commonly referred to as EAAT-2 and GLT-1, to fully reuptake glutamate. Excitotoxicity has been suggested as one possible contributor to the multifactorial Amyotrophic Lateral Sclerosis (ALS) pathophysiology. In fact, the sole FDA-approved treatment, Riluzole, is thought to work by decreasing excitotoxicity. Early electrophysiological studies of transgenic mouse models suggested that embryonic ALS mouse motoneurons are hyperexcitable, but more recent studies of adult motoneurons have suggested that ALS-affected motoneurons may be hypoexcitable. We hypothesize that these findings could be explained by temporal changes in excitability that are a function disease progression. We perform a meta-analysis of quantifiable excitability measures in the SOD1 G93A (superoxide dismutase-1 glycine 93 to alanine) transgenic ALS mouse model and compare the results to age-matched wild type mice. Included measures comprise assessments of glutamate concentration, glutamate transports (GLT-1 and EAAT2), receptor activation (NMDA, AMPA, GABA), calcium concentration, acetylcholine concentration, and compound muscle action potential (CMAP) amplitude. Our results reveal that many of the assessed measures fluctuate over the course of disease progression, likely in an attempt to maintain homeostasis. Specifically, there is a distinct change that occurs prior to disease onset, which results in an apparent shift from hyperexcitability to hypoexcitability.

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Poster

069. Motoneuron Disease

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 69.23/O47

Topic: D.13. Motor Neurons and Muscle

Support: NIH NINDS NS077863

Title: Hyperexcitability in synaptic activity as a component of excitotoxicity in adult mouse model of amyotrophic lateral sclerosis

Authors: *C. HECKMAN[1], M. JIANG[2];
[1]Dept. of Physiol., [2]Northwestern Univ., Chicago, IL

Abstract: Hyperexcitability likely contributes to the degeneration of spinal motoneurons (MNs) in amyotrophic lateral sclerosis (ALS). Studies thus far indicate that there is no hyperexcitability in the intrinsic electrical properties of MNs in the adult mutant SOD1 mouse model of ALS (high copy number, mSOD1G93A). In this study, we obtained two types of measurements, reflexes to assess net system behavior and intracellular recordings in motoneurons to assess single cell behaviors. All studies were carried out in an adult *in vitro* preparation of the sacral spinal cord of mSOD1G93A mice and their wild type controls (age range: 50 - 90 days). At the system level, we found that the maximum compound action potentials (coAPs) evoked in ventral roots by short train stimulation of corresponding dorsal roots were similar between the mSOD1 G93A mice and their nontransgenic (NG) littermates. There was however substantial depression of coAPs during the course of the train stimulation and this depression was significantly reduced in the mSOD1G93A mice, suggesting presence of a net increase in potency of sensory input to MNs. At the single cell level, intracellular recordings revealed no changes in the amplitudes of excitatory postsynaptic potentials (EPSPs) in the mSOD1G93A MNs. In contrast, short lasting poly-EPSPs coupled to both oscillations and depolarizations (PEODs) in membrane potential were significantly more common and could be produced with significantly lower stimulation intensity in mSOD1G93A MNs. These PEODs depended on spinal network and activation of NMDA receptors and were associated with increased levels of spontaneous activity. No significant changes were found in intrinsic properties of these mSOD1G93A MNs. Taken together, these findings revealed hyperexcitability in synaptic mechanisms in MNs of mSOD1G93A mice, which could contribute to the excitoxicity in ALS.

Disclosures: C. Heckman: None. **M. Jiang:** None.

Poster

070. Gait and Posture: Afferent Control

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 70.01/O48

Topic: D.16. Posture and Gait

Support: NICHD 5T32HD055180

NINDS 5R01NS069655

Title: Split-belt treadmill adaptation in trans-tibial amputees

Authors: *B. P. SELGRADE, M. E. TONEY, Y.-H. CHANG;
Applied Physiol., Georgia Inst. of Technol., Atlanta, GA

Abstract: A common paradigm used to study locomotor adaptation is split-belt treadmill walking, in which the subject adapts to walking with each leg moving at a different speed. Recent work indicates that this adaptation is driven by optimizing metabolic power (Finley et al, 2013). The changes in walking mechanics that drive these metabolic changes, however, are not fully understood. Previously, we showed that, as subjects begin walking in the split-belt condition, the single support (SS) work by the leg on the fast belt increases immediately. As subjects adapt to split-belt walking, reductions in total mechanical work on the center of mass per stride mirror the changes in metabolic power. Notably, there is a decrease in positive work by the SS leg and a corresponding increase in positive work done during the step-to-step transition (STS). This shift in mechanical power generation from SS to the trailing leg in STS agrees with the general principles of dynamic walking. However, it is unknown how the loss of the ankle joint, the primary power producer, would impact this split-belt locomotor adaptation. Therefore, we examine changes in mechanical work done by trans-tibial amputees during split-belt adaptation. We hypothesized that, for both amputees and controls, total stride work and positive work during SS would decrease as subjects adapted to split-belt treadmill walking, while positive work during STS would increase. Eight trans-tibial amputees and 8 matched control subjects participated in this study. Subjects walked with the prosthesis (non-dominant leg for controls) on a belt moving at 150% of preferred walking speed (PWS) and the intact leg on a belt moving at 75% PWS. We calculated mechanical power and work done by each leg on the center of mass. For control subjects, the hypotheses were supported. Total stride work decreased between early and late adaptation, as did positive work done during SS, particularly for the leg on the fast belt. Positive work done during STS increased over this time. For amputees, the hypotheses were partially supported. Total stride work decreased from early to late adaptation, but positive work during STS did not increase over this time. Thus, amputees only slightly reduced SS work between early and late adaptation. For controls, the fast trailing leg provided most of the positive work during STS. In contrast, amputees were limited in the push-off work from their prosthetic leg on the fast belt. Rather than relying on ankle work during STS, amputees rely on the less economical strategy of hip work during SS, which could represent a generalized strategy that amputees use in novel walking conditions.

Disclosures: B.P. Selgrade: None. **M.E. Toney:** None. **Y. Chang:** None.

Poster

070. Gait and Posture: Afferent Control

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 70.02/P1

Topic: D.16. Posture and Gait

Support: NIH Grant 5R01NS069655

Title: Effects of decreased contralateral afferent feedback on ipsilateral motor output during pedaling

Authors: *Y.-H. CHANG, T. L. NORMAN;
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Abstract: The neuromuscular control of locomotion is modulated by peripheral sensory feedback. It is not understood exactly how sensory feedback modulates locomotion, or to what functional effect. In human pedaling studies, Ting and colleagues (1998, 2000) showed that an increase in contralateral extension force elicits a decrease in ipsilateral flexor motor output during the pedaling upstroke, suggesting contralateral extensor muscle afferents (e.g., force-dependent Ib afferents from golgi tendon organs) inhibit the ipsilateral flexor muscles. A similar response was shown in a reduced rat preparation during swing phase of walking (Hayes et. al, 2012; Hochman et. al, 2013). We hypothesized that ipsilateral flexor motor output would increase when contralateral plantarflexor afferent feedback was decreased. To test this hypothesis we employed the use of a custom cycle ergometer with mechanically decoupled cranks and instrumented pedals. We collected preliminary data on six subjects participating in the IRB-approved protocol (males, 5; females 1). On day one, subjects underwent training on the decoupled cycle ergometer. On day two, subjects completed 45-second pedaling trials where the left (recipient) leg always pedaled. A baseline trial of bilateral pedaling was collected and used to compare to decreased afferent feedback conditions. We decreased contralateral plantarflexor afferent feedback by imposing ischemic deafferentation to the right (donor) leg. A cadence of 60 rpm was maintained for all conditions. We collected kinematics (120Hz, Vicon), kinetics (300Hz, Kister), and electromyographic data (1080Hz, Noraxon) from tibialis anterior, soleus, medial gastrocnemius, biceps femoris long head, rectus femoris, and gluteus maximus muscles. Mean muscle activations of four functional pedal cycle quadrants were analyzed: Q1. anterior transition; Q2. Extension; Q3. posterior transition; Q4. flexion. After 20 minutes of right (donor) leg ischemic deafferentation, the left (recipient) rectus femoris and tibialis anterior activations were not significantly different from baseline during Q4. flexion (96±13% baseline activation, $p=0.960$ and 83±31% baseline activation, $p=0.244$, respectively). In the subsequent pedaling phase, Q1. anterior transition, the left (recipient) left rectus femoris activation was significantly

lower than baseline (58±21% baseline activation, p=0.005) and tibialis anterior activation trended toward a significant decrease from baseline (67±39% baseline activation, p=0.092). Our preliminary findings do not support our hypothesis and require further investigation.

Disclosures: Y. Chang: None. **T.L. Norman:** None.

Poster

070. Gait and Posture: Afferent Control

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Topic: D.16. Posture and Gait

Support: Det Obelske Familiefond

SparNord Fonden

Title: Interlimb reflexes following ipsilateral knee joint rotations are suppressed in an unstable walking environment

Authors: *A. J. STEVENSON[1], S. S. GEERTSEN[2,3], T. SINKJÆR[1,4], J. B. NIELSEN[2,3], N. MRACHACZ-KERSTING[1];
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Abstract: Interlimb reflexes play an important role in human walking, such as when dynamic stability is threatened by external perturbations or changes in the walking surface. For example, we have previously shown that interlimb reflexes in the contralateral biceps femoris (cBF) following ipsilateral knee (iKnee) extension rotations during walking contribute to slowing the forward progression of the body in order to maintain dynamic stability following the perturbation (Stevenson et al., 2015). However, little is known about how such interlimb reflexes are modulated in an unstable walking environment. Based on experiments investigating intra- and interlimb cutaneous reflexes and soleus H-reflexes (Llewellyn et al., 1990; Haridas et al., 2005; 2006; Krauss and Misiaszek, 2007), we hypothesized that the amplitude of interlimb reflexes following iKnee perturbations is altered when walking in an unstable environment. To test this, interlimb reflexes were elicited in participants (n = 6) by either iKnee extension rotations during the late stance phase (50%) of the gait cycle or iKnee flexion rotations during the mid-swing

phase (80%). The iKnee perturbations were applied while the participants walked normally on a treadmill (stable condition), or while random increases or decreases in velocity were applied to the treadmill (unstable condition). The abrupt treadmill velocity changes were applied every 3-8 steps, included three different velocity changes between ±1.12 m/s, and lasted for 1.5 seconds. They were strong enough to cause instability, but not enough to result in a fall. In the unstable condition, the iKnee perturbations were applied in steps without treadmill velocity changes. During normal walking, iKnee extension perturbations elicited facilitatory interlimb reflexes in the cBF and contralateral soleus (cSOL) muscles with onset latencies of 81 and 92 ms, respectively, while iKnee flexion perturbations elicited reflexes in the cBF (facilitatory) and cSOL (inhibitory) with onset latencies of 66 and 85 ms, respectively. The cBF reflex amplitudes were significantly suppressed (P's < .05) in the unstable walking condition following both types of iKnee perturbations, while the cSOL reflexes remained unchanged. Consistent with previous studies, these preliminary results suggest that challenging dynamic stability during walking leads to specific changes in reflex amplitudes that are not related to a generalized change in reflex excitability. Descending cortical influences likely contribute to the specific modulations based on the environmental demands.

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Poster

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Program#/Poster#: 70.04/P3

Topic: D.16. Posture and Gait

Title: Postural responses to various frequencies of vibration of the triceps surae and forefoot sole during quiet standing

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Abstract: Sensory information from lower-leg muscles and soles of the feet is important for the perception of standing position. When a subject is standing with eyes closed, vibration of the triceps surae or forefoot soles at 20-150 Hz induces backward-lean of the body for most subjects. The postural response to vibration is thought to occur as a compensatory response to the

illusionary perception created via the sensory reference system. In quiet standing, the center of pressure sways within ± 1 cm at very low frequencies, mainly ≤ 2 Hz. It may be assumed that the resulting low-frequency oscillations in sensory information from the lower-leg muscles and foot soles are involved in the organization of the sensory reference frame in quiet standing. However, vibration frequencies lower than 20 Hz have not been previously investigated. In present study, we investigated the postural responses induced by vibration of the triceps surae and/or forefoot sole at various frequencies (1-60 Hz) to determine the role of somatosensory input to the sensory reference system in quiet standing. Thirteen participants participated in the full experiment. The experiment consisted of two sessions: 1) single vibration and 2) simultaneous vibration. Vibration at high and low frequencies induced backward- and forward-lean responses, respectively. The lowest vibration frequencies (defined as B-LF) inducing backward-lean responses were 15-55 Hz for the triceps surae and 16-60 Hz for the forefoot sole. The highest frequencies (F-HF) inducing forward-lean responses were 3-18 Hz for the triceps surae and 1-20 Hz for the forefoot sole. When vibration was simultaneously applied to the triceps surae and forefoot sole at F-HF, no response was induced in 70% of trials. A forward-lean response was induced in the remaining 30% of trials. Simultaneous vibration of the triceps surae and forefoot sole at B-LF induced backward-lean responses in all trials. All postural responses occurred 0.5-4.3 s after vibration onset. Postural responses to high-frequency vibration conceivably occur as a compensatory movement to the illusionary perception that standing position is deviating forward from quiet standing, which must be a reference position. Postural responses to low-frequency vibration possibly occur to equalize the positional information that received from the triceps surae and the forefoot sole. Both postural responses are likely to involve the sensory reference system, which is located in the supraspinal nervous system.

Disclosures: **M. Naka:** None. **K. Fujiwara:** None. **N. Kiyota:** None.

Poster

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Topic: D.16. Posture and Gait

Support: NIH R01 HD053367 to NSB

Title: Flexor and extensor muscle recruitment during repetitive leg movements in chick embryos

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Abstract: Prior to hatching, chick embryos spontaneously produce repetitive limb movements (RLMs). RLMs are a developmental precursor to walking after hatching. They exhibit features of walking, including alternating leg flexor and extensor muscle bursts at step cycle frequencies. Frequently, however, flexor activity is rhythmic for many cycles, whereas extensor activity often drops out for one or more cycles. We hypothesize that during RLMs the spinal pattern generator for stepping more readily recruits leg flexor muscles than extensors. The purpose of this study is to determine if flexor and extensor muscle recruitment differs during RLMs. We recorded electromyography (EMG) and kinematics during spontaneously produced leg movements at embryonic day 20. Leg EMG was recorded either ipsilaterally (hip and ankle flexor and extensor muscles) or bilaterally (ankle flexor and extensor muscles). RLM EMGs were identified by 3 criteria: 4 or more consecutive rhythmic bursts in at least 1 muscle; burst cycle frequencies at 1-10 Hz; and accompanied by leg movements. To test for differences in flexor and extensor recruitment, RLM burst counts, burst durations and burst amplitudes for antagonist muscle pairs of the hip or ankle were compared within embryo (N=21, Wilcoxon signed rank test). Burst amplitudes were normalized to the maximum value within muscle to estimate the recruitment differences between antagonist muscle pairs. Results indicated that flexor bursts outnumbered extensor bursts in 35 of 37 antagonist muscle pairs, and the difference was significant ($p < 0.001$). Flexor bursting in the absence of extensor bursting commonly occurred, whereas extensor bursting in the absence of flexor bursting was rare. Burst duration and normalized burst amplitude were similar for antagonistic muscle pairs. However, flexor peak burst amplitude was lowest if the extensor was not recruited during an RLM, and greatest if the extensor participated in all cycles of an RLM ($p < 0.006$). Our results provide evidence that flexor muscle recruitment and extensor recruitment differ during RLMs. Results suggest that flexor muscles are preferentially recruited at lower levels of neural drive, and extensors are recruited as drive increases, consistent with our hypothesis. Our findings also suggest that the spinal pattern generator readily recruits flexor motor pools independent of extensor pools, but rarely recruits extensor pools independently. However, when both antagonist motor pools are recruited, similar burst durations and amplitudes are produced. These findings lead us to conclude that recruitment threshold and burst generation are separately controlled during RLMs in chick embryos.

Disclosures: **S. Sun:** None. **N.S. Bradley:** None.

Poster

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Topic: D.16. Posture and Gait

Support: NSERC (Canada) Grant

Title: Early activation of ankle muscles following light touch displacement at the fingertip during treadmill walking

Authors: T. SHIVA[1], J. FORERO[1], *J. E. MISIASZEK[2];
[1]Fac. of Rehabil. Med., Univ. of Alberta, Edmonton, AB, Canada; [2]Univ. Alberta, Edmonton, AB, Canada

Abstract: Recently, we demonstrated that unexpected displacement of a touch reference during eyes-closed standing can produce rapid activation of leg muscles consistent with an automatic postural response, despite the absence of a mechanical disturbance to balance. However, these responses were relatively small, not expressed in all participants and not expressed in subsequent trials. We speculated that the touch-evoked reaction would be more pronounced when the touch cue had a greater functional relevance. Light touch at the fingertip has been shown to allow individuals to walk on a treadmill in the absence of vision. Light touch also affects the amplitude of corrective reactions to unexpected balance disturbances during treadmill walking. Therefore, we hypothesized that unexpected displacement of a touch reference during eyes-closed treadmill walking would result in pronounced corrective reactions, comparable to reactions produced following mechanical balance disturbances during walking. To test this hypothesis, participants walked on a motorized treadmill with their vision occluded and lightly touching a rod with their right index finger. The rod was positioned in front of the participants at shoulder width such that their upper arm was vertical and their forearm was horizontal with a neutral wrist. After several minutes of walking with eyes-closed and touching the stable rod, a rapid displacement (1.25 cm, 12.5 cm/s peak velocity, 187.5 cm/s/s peak acceleration) of the rod was applied at right heel-strike. The direction of the initial displacement (forwards/backwards) was randomized across participants. Nine additional displacements in the same direction were then applied at heel-strike at unexpected intervals. Electromyographic recordings from 4 leg and 4 arm muscles were sampled along with joint kinematic data from the elbow, knee and ankle. Rapid displacement of the touch reference resulted in short-latency (<100 ms) responses in the muscles of the leg. Evoked responses were particularly pronounced in tibialis anterior following forward displacement of the touch reference. Response amplitudes were largest for the initial, unexpected displacement. Responses continued to be evoked throughout the experiment in most participants. Responses in the arm muscles were rarely observed. Following the experiment some participants reported that they believed changes to the speed of the treadmill were introduced, rather than a displacement of the touch reference. These results indicate that cutaneous input from a single fingertip can initiate a profound and robust adaptation to ongoing walking, consistent with a balance corrective response.

Disclosures: **T. Shiva:** None. **J. Forero:** None. **J.E. Misiaszek:** None.

Poster

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Topic: D.16. Posture and Gait

Support: NIH Grant EB 012855

NIH Grant HD32571

Title: Modulation of afferent feedback from paw pad afferents affects interlimb coordination and adaptation to split-belt treadmill locomotion in the cat

Authors: H. PARK, R. MEHTA, S. P. DEWEERTH, *B. I. PRILUTSKY;
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Abstract: Split-belt treadmill locomotion has been used to study the organization of interlimb coordination in cats (Forssberg et al. 1980) and locomotor adaptation in humans (Dietz et al. 1994). These studies have revealed some neural constraints on left-right and fore-hind limb coordination and brain structures contributing to locomotor adaptation. Motion-dependent sensory feedback is important for interlimb coordination and locomotor adaptation. For instance, differences in visual information between overground and treadmill locomotion appear to influence interlimb coordination in cats (Blaszczyk, Loeb, 1993) and the amount of adaptation in humans (Torres-Oviedo, Bastian, 2010). Here we investigated the role of cutaneous feedback from cat paws on interlimb coordination and split-belt treadmill adaptation in adult cats. Four adult cats were implanted with stimulating nerve cuff electrodes on distal tibial and sural nerves innervating skin on plantar surface of paw and foot. A recording cuff electrode was implanted on sciatic nerve. Thresholds for activation of cutaneous sensory fibers to single pulse and train of pulses of electrical stimulation were established in each animal by recording the responses in the sciatic nerve. Several locomotor conditions were investigated including: (1) tied-belt walking with the same speed of left and right belts (LBS=RBS=0.4 m/s), (2) tied-belt walking at 0.4 m/s with the right forepaw and hindpaw anesthetized by lidocaine injections, (3) tied-belt walking at 0.4 m/s with low-intensity train stimulations of distal tibial or/and sural nerves (</= 1.2T, duration </= 250 ms) triggered by right hindpaw contact with the ground, (4) split-belt walking (LBS=0.4 m/s, RBS=0.6 m/s and RBS=0.8 m/s), (5) split-belt walking with the right forepaw and hindpaw anesthetized, and (6) split-belt walking with the right forepaw and hindpaw anesthetized and with stimulations of sensory nerves during stance. Symmetry between right and left step/stride length and relative phase between fore- and hindlimbs were affected by

manipulation of cutaneous feedback from paw pad afferents. For example, paw pad anesthesia increased step length symmetry between the left and right sides but increased ground reaction forces on the affected side. The combination of anesthesia and sensory nerve stimulation reduced pacing and variability of the stride length symmetry index during split-belt walking. We concluded that interlimb coordination and adaptation to split-belt walking depends on sensory feedback from paw pad cutaneous afferents.

Disclosures: **H. Park:** None. **R. Mehta:** None. **S.P. DeWeerth:** None. **B.I. Prilutsky:** None.

Poster

070. Gait and Posture: Afferent Control

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Program#/Poster#: 70.08/P7

Topic: D.16. Posture and Gait

Title: Gait characteristics of children walking barefoot and with socks

Authors: *C. W. CHAU, L. ALBERT, A. AUFIERO, L. MOCK, K. WARD;
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Abstract: The aim of this study is to compare the characteristics of spatiotemporal gait parameters in typically developing 4 and 5 year old (yo) children while walking barefoot and with socks, at a self-selected regular and fast walking speed. Twenty children (10 male and 10 female) participated in this study. All subjects walked on the GAITRite® (CIR System Inc. NJ), a portable carpeted walkway (3X14ft) embedded with electronic pressure sensors that record footprints. Each subject completed four trials of the four walking conditions (barefoot and with socks at regular speed and fast speed) in a randomized order. Spatiotemporal gait parameters were recorded and analyzed with the GAITRite® system which included velocity, cadence, step and stride length, cycle time, stance and swing phase duration, single and double support percentages. Paired t-tests were used to compare gait parameters while walking barefoot and with socks, for both regular and fast walking speeds. The preliminary results showed that spatiotemporal gait parameters were comparable between walking barefoot and with socks at a regular walking speed, but different when walking at a fast speed. During fast walking with socks as compared to barefoot, there was 1) a statistically significant increase in cadence (+10.9 steps/min,) and decrease in cycle time (-30ms); 2) a slight, but statistically insignificant, increase in velocity (+6.0 cm/sec), swing (+0.5%), and single support (+0.6%); and 3) a decrease in stride length (-2.1 cm), stance (-0.4%), and double support (-0.6%). The foot length and width are comparable between walking barefoot and with socks. Similar but slightly greater differences in

cycle time (-3.2 ms), step length (-0.5 cm), stride length (-0.6 cm), and cadence (+3.1 steps/min) was observed in 5-yo children as compared to 4-yo children during fast walking with socks. These findings suggest that children walking at a fast speed with socks displayed a more unstable gait than when walking barefoot. It is possible that socks may alter cutaneous input and also increase slippage especially during fast walking. During regular walking speed, similarities in spatiotemporal gait parameters between walking barefoot and with socks was consistent with literature suggesting that cutaneous input exerts minimal effect on undemanding locomotion. Greater changes in 5-yo as compared to 4-yo children suggest that developmental maturation may play a role in the ability to adapt the locomotor pattern to different conditions.

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Poster

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FRQS Salary awards JSR CM

CIHR Fellowship JB

CIHR Salary award CM

IRSST Scholarship JB

Title: Development of a reliable proprioception measure during gait using a robotised ankle-foot orthosis and its relationship with static and dynamic balance

Authors: *J. BOUFFARD[1,2], A. FOURNIER-BELLEY[2,1], D. MORIN[1], C. MERCIER[1,2], J.-S. ROY[1,2], L. J. BOUYER[1,2];
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Abstract: Background: Proprioception is a crucial property of the sensorimotor system providing feedback for motor control and learning. Most proprioception measures currently used

evaluate the ability of individuals to detect movement, position or force imposed to their limb in an otherwise static state. However, proprioceptive capacities in dynamic tasks such as gait are much more complex than what is evaluated by such tests as the sensory signals coding for movement, position and force interact together and with the expected feedback related to the ongoing movement. **Objective:** To develop and validate an innovative evaluation of proprioceptive capacities during gait using a robotised ankle-foot orthosis. **Method:** Twenty-five healthy individuals were tested twice in a 3 to 7 days interval. On each lab session, participants walked on a treadmill using a robotised ankle-foot orthosis during two ~6 minutes periods. During those walking periods, force perturbations of varying amplitudes resisting participants' ankle dorsiflexion during swing were applied every 3 to 7 strides. Participants were asked to press on a handheld switch whenever they detected a perturbation. They also performed the Star Excursion Balance Test (SEBT) and the Balance Error Scoring System (BESS) as ankle proprioception is considered to influence balance. **Data analysis:** The peak ankle error caused by the perturbations, i.e. the maximal difference between ankle movements of a perturbed stride and of the unperturbed strides, was calculated for each perturbed stride. A sigmoid curve presenting the detection probability in relation to the peak ankle error caused by the perturbations was then computed for each lab session. The peak ankle error with a detection probability of 50% was considered as the detection threshold. The intraclass correlation coefficient (ICC) and minimal detectable change (MDC_{95}) were calculated to determine test-retest reliability of detection threshold. Pearson correlation coefficients between detection threshold and balance scores were calculated. **Results:** The mean detection threshold of the participants was 5.04° (range 1.04°-9.14°). The detection threshold has a good reliability with an ICC of 0.79 and a MDC_{95} of 2.39. The detection threshold correlates moderately with the SEBT (dynamic balance, r=0.61) but not with the BESS (static balance, ns). **Conclusion:** The newly developed measure provides a reliable method to assess proprioception. The correlation with a dynamic balance score supports its validity. This innovative measure will offer important opportunities in gait rehabilitation and in basic motor control research.

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Poster

070. Gait and Posture: Afferent Control

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Topic: D.16. Posture and Gait

Support: EB012855

NS055976

Title: Comparing the contribution of length and force feedback to ankle extension during stance in the treadmill trained spinal cat

Authors: *A. J. KRUPKA[1], D. HIGGIN[2], B. I. PRILUTSKY[3], A. N. KLISHKO[3], I. RYBAK[2], M. A. LEMAY[1];
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Abstract: Treadmill training of spinalized animals promotes recovery of locomotor function and typically focuses on walking on horizontal surfaces, a model that fails to include the difficulties faced by patients with spinal cord injury (SCI) operating in a community environment. The purpose of this study was to investigate the contribution of various sensory modalities of the ankle extensor muscles and paw cutaneous feedback in the adaptations to walking on graded surfaces in spinal cats. Eight cats were utilized in these experiments. Four cats (group 1) underwent lateral gastrocnemius/soleus (LGS) self-reinnervation in the right hindlimb 3 months prior to spinalization while the remaining four (group 2) did not undergo any surgery prior to spinalization. After collection of pre-transection kinematics data, all cats underwent complete spinal cord transection. After transection, cats were trained to step on a treadmill at a speed of 0.4m/s until their locomotor performance plateaued, which took from 4 to 12 weeks. Once stepping proficiency was attained, kinematic recordings were taken for all cats stepping on a treadmill at 0.4m/s on flat, incline, and decline surfaces (10° and 25°). Group 2 cats then underwent right hindlimb deafferentation at the L7-S1 levels. Daily training continued post-deafferentation and kinematics were recorded for these cats 1, 8, and 12 weeks post-deafferentation. Animals that underwent LGS self-reinnervation before spinal transection recovered plantar weight-bearing stepping on the treadmill for flat, ±10°, and ±25° sloped surfaces. These animals showed limited differences in the kinematics of the two hindlimbs, suggesting that ankle Ia afferent feedback of these major ankle extensors, which is permanently lost after self-reinnervation (Cope et al 1994), has minimal effects on locomotor recovery in spinal animals. In animals that underwent an L7-S1 deafferentation, affecting both length and force feedbacks of the triceps surae muscles as well as the footpad cutaneous feedback, a persistent deficit in ankle plantar-flexion prevented recovery of stepping on flat or graded surfaces. These results are consistent with modeling studies (Markin et al, 2015) showing that muscle and cutaneous load-sensitive afferents play a greater role in the production of extensor torque at the ankle during stance than length- and velocity-sensitive afferents.

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Poster

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Topic: D.16. Posture and Gait

Support: NSERC DG 250348

Title: The effects of plantar vibration stimulation on lower limb muscle activity during stepping

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Abstract: The somatosensory system has been shown to play a significant role in balance control in conjunction with vision and the vestibular system. Evidence has shown that manipulation of the mechanoreceptors, on the plantar surface of the foot, has a direct effect on balance control. By manipulating these receptors with vibration, researchers are capable of stimulating the mechanoreceptors within the foot to better understand the role that vibration plays in the potential enhancement of balance control via measurement of its effect on muscle activity within the lower limb. A total of eight healthy young participants were recruited for this study. The participants were asked to complete a voluntary stepping task while undergoing three different vibration conditions. The stepping task involved a three-step forward gait movement that terminated with the feet side-by-side. The participants performed this task with no vibration, subthreshold (90% of perceptual threshold, white noise) vibration (delivered throughout the trial), and suprathreshold (3x perceptual threshold, 60 Hz) vibration on the plantar surface of the feet (triggered by stepping onto the first force plate (~10N force level)). The vibration was placed under the first metatarsal and the heel of the right foot. The subsequent muscle activity during these tasks was recorded and analyzed to determine changes in onset, magnitude and reflex activity of muscle activity, occurring just prior to the second step of the gait termination task, within the three experimental conditions. The results of this study indicated that there was a significant decrease in the magnitude of the medial gastrocnemius activity for both the subthreshold and suprathreshold vibration conditions ($p<0.05$). Furthermore, it was observed that the medial gastrocnemius also demonstrated an earlier onset timing of activity for both vibratory conditions in comparison to the no vibration condition ($p>0.05$). Reflex analysis indicated that the introduction of vibration demonstrated facilitation of gastrocnemius muscle activity within short (150ms) and long (>250ms) latency time frames. Therefore, this study demonstrates that the use of vibration stimulation is modulating muscle activity (magnitude, timing, and reflexes) within the lower limb. However, the direct connection between vibration and lower limb muscle

activity hasn't been fully investigated. Thus further research needs to be completed in order to identify the specific relationship between muscle activity and vibration in balance control.

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Poster

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Topic: D.16. Posture and Gait

Support: FAPESP (2012/09321-1)

Title: The influence of vision and foam pad on the coherence analysis between the center of pressure and light touch tangencial force during quiet stance

Authors: C. R. SILVA[1], F. H. MAGALHÃES[2], *A. F. KOHN[3];
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Abstract: Removing visual information in a healthy adult population typically leads to an increase in mean sway amplitude. If this change is coupled with an unstable surface, the postural sway can be even greater. The tactile information from a fingertip lightly and voluntarily touching a fixed surface (active touch) has been suggested to be able to reduce postural sway. So, the aim of the present investigation was to evaluate whether the absence of vision and the unstable support surface (Airex balance pad - FP) change the relationship between the center of pressure (COPAP) and fingertip forces (Fx) during quiet standing. Sixteen healthy subjects were requested to keep a quiet stance during upright stance in four conditions: 1) V = vision - no FP; 2) VFP = vision with FP; 3) D = no vision - no FP and 4) DFP = no vision with FP. In all conditions the subjects performed the tests touching a mini force platform with the tip of the index finger (vertical force < 1 N). The four experimental conditions were presented in a randomized order. The subject performed 4 trials, each lasting 90 s, for each of the two experimental conditions. A resting period of ~120 s between trials was allowed to avoid fatigue (subject sat in a comfortable armchair placed next to the force plate). The COPAP was measured using a force platform. The coherence analysis was performed between Fx and COPAP signals for all conditions (FxCOP). There was no significant coherence in the V and D condition. Subjects standing on the FP showed a significant anti-phase coherence for a frequency range from approximately 0.05 to 0.3 Hz and 0.01 to 0.6 Hz for VFP and DFP, respectively. These

results suggest that vision does not provide influences on FxCOP in conditions without FP (V versus D). However, in FP conditions (VFP versus DFP) the absence of vision shows an anti-phase pattern in a greater range of frequency. The influence of the FP (V versus VFD and D versus VDF) in the analysis shows a change in the interactions between the COPAP and the Fx, with significant coherence at low frequencies (no presented in FP-free condition). When standing on foam, the information from the mechanoreceptors of the feet is believed to become less reliable, changing the discharge patterns from the receptors, which increases postural sway and changes the standing strategy. The results suggest possible changes in biomechanic factors (stiffness, viscoelasticity, muscle activity pattern) involved in the control of during quiet stance. Thus, further analyses of other variables such as muscle EMGs and other body angles are under way to provide a more global understanding of the possible mechanisms behind postural control of humans standing on unstable surfaces.

Disclosures: C.R. Silva: None. **F.H. Magalhães:** None. **A.F. Kohn:** None.

Poster

070. Gait and Posture: Afferent Control

Location: Hall A

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Topic: D.16. Posture and Gait

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Helsinki Biomedical Graduate Program of Helsinki University

Title: Cortical recovery rate of proprioceptive responses to passive finger movements

Authors: E. SMEDS, *R. K. HARI, H. PIITULAINEN, M. BOURGUIGNON, V. JOUSMÄKI; Dept. of Neurosci. and Biomed. Engin., Aalto Univ., Espoo, Finland

Abstract: Objective: An increase in stimulus repetition rate typically results in attenuation of cortical evoked responses; the longer the latency of the response, the stronger the effect. Previous

research has shown that this pattern applies over a wide range of sensory modalities. Proprioceptive responses, however, require further characterization that would benefit both basic and clinical research. Therefore, we here studied the stimulus-rate dependence of cortical responses to passive finger movements. **Methods:** We measured magnetoencephalographic (MEG) signals from 15 healthy adults during passive intermittent extension and flexion movements of the right index finger (306-channel whole-scalp device by Elekta Oy; passband 0.1-330 Hz; sample frequency 1000 Hz). The motion occurred mainly at the metacarpophalangeal joint, which moved between a neutral and a slightly over-extended position. We here present data only for the extension movements. A specially designed pneumatic movement stimulator (http://dx.doi.org/10.1016/j.neuroimage.2015.03.006; Piitulainen et al., Neuroimage 2015) elicited passive movements at interstimulus intervals (ISIs; times between consecutive extensions) of 0.5, 1, 2, 4, 8, and 16 s. The ISI remained unchanged in each measurement condition, and the order of conditions was randomized for each subject. The movements were monitored with an accelerometer attached to the fingernail. MEG signals were averaged time-locked to the movement onsets and low-pass filtered at 40 Hz. The peak amplitudes of the averaged responses were determined from the gradiometer vector sum at the site of the maximum response (fixed site for all ISIs of a single subject). An exponential model of the form $A(ISI) = A_{plateau}(1 - e^{-ISI/\tau})$ was fitted to the response amplitude (A), where A_{plateau} is the saturation amplitude, and τ is the recovery constant of the response. **Results:** The main response to extension movements peaked at ~80 ms, with sources in the contralateral primary somatosensory cortex. Responses were absent or weak at the shortest (0.5-s) ISI, and increased in amplitude as the ISI was extended, reaching a plateau at the 8-s ISI. The exponential model yielded a recovery constant τ = 1.2 s. This dependence implies that, for passive finger extension movements, a ~1.5-s ISI would maximize the signal-to-noise ratio of the cortical response in a fixed measurement time. **Conclusions:** Our findings can be exploited to develop efficient proprioceptive stimulation protocols. Cortical responses to accurately timed passive movements provide a novel quantitative tool for assessment of human proprioception, which currently lies beyond the reach of clinical tests.

Disclosures: E. Smeds: None. **R.K. Hari:** None. **H. Piitulainen:** None. **M. Bourguignon:** None. **V. Jousmäki:** None.

Poster

071. Cortical Planning and Execution: Electroencephalogram

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 71.01/P13

Topic: D.17. Voluntary Movements

Title: Analysis of EEG microstates dynamics captures whole-brain states during reaching and grasping movements

Authors: ***E. PIRONDINI**[1], M. COSCIA[1], J. MILLÁN[2], D. VAN DE VILLE[3,4], S. MICERA[1,5];
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Abstract: Execution of complex movements, such as reaching and grasping, requires the integration of multisensory inputs by the human brain. Tactile and proprioceptive information are combined across brain areas and coordinated with eye, limb, and hand movements. This complex integration process requires the exchange of information between independent functional brain regions. Therefore, whole-brain functional data are necessary to visualize these processes and the underlying multi-functional organization of brain activity. Analysis of electroencephalographic (EEG) microstates could be used to identify the recurrent organization of the whole-brain activity that might play a role in the sensory-motor integration processes of the human cortex. Several studies have shown that brain activity during resting-state is organized in meta-stable states characterized by periods of coherent synchronized activation of neural networks, i.e., EEG microstates, that can be detected from EEG recording as polarity-independent topographic maps with a typical duration of 80-150 ms. These topographic maps could characterize different global brain states. In our preliminary work, we showed, in a small group of subjects, that analysis of EEG microstates reveals a recurrent organization of brain networks during movement execution. We here extended our previous investigation to pure reaching and reaching and grasping movements, separating movement phase and holding phase, in eight healthy subjects. We found that the EEG microstate repertoire for execution of voluntary movements was richer than for resting state, although the four prototypical microstates of resting state were still present in both settings, but with a reduced duration during movement execution. In particular, we found two new topographies common in different motor tasks, and one task-specific EEG microstate, which disappeared during the holding phase. The EEG microstates related to movement execution were event-related and the movement network activity was located in pre-motor and posterior parietal cortex. Intracranial sources were estimated using eLORETA on an average brain model. Interestingly, during the movement planning phase, we observed a significantly increased occurrence of the EEG microstate that has been previously linked to the executive-control network. In conclusion, our results provide evidence of movement-specific EEG microstates that capture whole-brain states and that are located in the fronto-parietal network typically involved in voluntary movements. This analysis helps

understanding how sensory-motor-control strategies are implemented in the central nervous system.

Disclosures: **E. Pirondini:** None. **M. Coscia:** None. **J. Millán:** None. **D. Van De Ville:** None. **S. Micera:** None.

Poster

071. Cortical Planning and Execution: Electroencephalogram

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 71.02/P14

Topic: D.17. Voluntary Movements

Title: Pain-related suppression of beta oscillations facilitate movement initiation

Authors: ***S. COOMBES**, E. OFORI, J. CHUNG, G. MISRA;
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Abstract: How pain affects movement is a subject of extensive research. Several electroencephalography (EEG) studies on the effect of pain on movement have made the onset of pain and onset of movement either concurrent or dependent. The goal in the current study was to determine the neural correlates of how ongoing pain influences the initiation and execution of movement. EEG studies of pain have demonstrated a pain-induced reduction in power in the alpha and beta bands. EEG motor studies have also demonstrated a movement-induced reduction in power in the same frequency bands, which suggests that pain may prime the motor system and facilitate movement. To test this hypothesis, healthy subjects performed a visually guided ballistic elbow flexion task during a 4 second long thermal pain stimulus while we recorded EMG signals from the arm and high density EEG signals from the scalp. We implemented a novel whole brain analysis based on independent component analysis, source localization, and measure projection analysis. We found that ongoing pain shortens reaction time significantly but does not affect movement velocity, acceleration and accuracy. The shortening was due to a reduction in pre-motor time and not due to a reduction in motor time. Spectral power in the beta band was source-localized to the contralateral sensorimotor cortex and was reduced significantly in the presence of pain. Further, beta power correlated positively with reaction time and pre-motor time but not with motor time. Hence, the mechanism of pain-induced shortening of reaction time cannot be peripheral but must be central in origin. Our findings demonstrate that a pain-related suppression of beta oscillations facilitates movement initiation.

Disclosures: **S. Coombes:** None. **E. Ofori:** None. **J. Chung:** None. **G. Misra:** None.

Poster

071. Cortical Planning and Execution: Electroencephalogram

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 71.03/P15

Topic: D.17. Voluntary Movements

Title: Effect of competition and prior probability of optional actions on beta-band EEG during preparation of movement

Authors: *Y. MATSUMOTO, A. FUJIKAWA, R. TAMAMURA, Y. KAKIMOTO, O. ARAKI;
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Abstract: In recent years, relations between neural oscillations and brain functions such as action and perceptions have been studied. It is known that the power of beta-band oscillatory decreases during the preparation and execution of movement. In the previous study (Tzagarakis et al., 2010), it is suggested that the more the number of possible actions is, the less the decrease of beta-band power from the baseline is. This modulation of beta-band decrease is thought to be due to the uncertainty of a target action. Here we questioned whether the uncertainty depends on the competition between plural optional targets or prior probability of each option. In both cases of alternative actions with equal or unequal prior probability and one target action with different probabilities, if only competition is dominant, the beta-band power decrease will remain constant, and if only prior probability is dominant, the beta-band power decrease will change depending on the probability. Thus, the purpose of our study is to verify these hypotheses, whether beta-band power decrease depends on the competition or the prior probability. We recorded electroencephalography (EEG) of 12 subjects during a reaching task, where the subject reaches for a target indicated by the color 1000 ms after candidates for target (red or green) are presented for 200 ms. We used five patterns of pre-cued possible target(s): one possible target with the probability of becoming a target 100%, 80% or 20%, and two possible targets with the prior probabilities 80%-20% or 50%-50%. We observed the effect of competition by comparing the data between one-target and two-targets, and the effect of prior probability by comparing those in different probabilities. The higher the prior probability is, the shorter the response time is when there is one target and there are two targets, which shows preparation for movement. When there are two possible targets (competitive condition), the decrease of beta-band power became smaller around the primary motor and premotor cortex during 400 ms before Go signal. On the other hand, in response to one possible target, the decrease of beta-band power remains constant in spite of prior probability. Consequently, these results show that the decrease of beta-

band power changes only under the competitive condition. Accordingly, the results suggest that the decrease of beta-band power is closely concerned with which way to go rather than whether to go or not during action preparation.

Disclosures: Y. Matsumoto: None. **A. Fujikawa:** None. **R. Tamamura:** None. **Y. Kakimoto:** None. **O. Araki:** None.

Poster

071. Cortical Planning and Execution: Electroencephalogram

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Topic: D.17. Voluntary Movements

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Title: Increased amplitude and earlier onset of EEG readiness potentials preceding voluntary actions associated with sensory consequences

Authors: *D. REZNIK, S. SIMON, R. MUKAMEL;
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Abstract: Self-generated, voluntary actions, are preceded by a slow negativity in the scalp EEG signal recorded from frontal regions (termed ‘readiness potential’; RP). This signal is mainly regarded as preparatory motor activity associated with the forthcoming motor act. However, it is not clear whether this neural signature is associated with preparatory motor activity or rather expectation of its associated sensory consequences. Here we recorded electroencephalography (EEG) data from 13 healthy subjects while they performed self-paced button-presses with their right index finger. In one condition (active) these button-presses triggered a sound while in a different condition (silent) they did not. Additionally, subjects passively listened to sounds delivered in expected timings (passive condition). Behaviorally, inter-press-intervals were

similar across the active and silent conditions. The EEG signal amplitude (locked to time of button press) was significantly more negative (500 ms prior to button press) in the active compared with silent condition. Importantly, no signal negativity was observed prior to sound delivery in the passive condition, and the increased negativity in the active condition was significant even after taking into account the activity preceding passive listening to the same sounds (passive condition). Furthermore, while the EEG signal negativity started 370 ms prior to silent button presses, in the active condition it started already 1430 ms prior to button presses. Thus differences in signal amplitude and temporal onset between active and passive conditions cannot be attributed to mere auditory expectation. Our results suggest that the motor activity preceding voluntary actions, encodes the expected auditory consequences and can modulate subsequent sensory-evoked activity in auditory cortex.

Disclosures: **D. Reznik:** None. **S. Simon:** None. **R. Mukamel:** None.

Poster

071. Cortical Planning and Execution: Electroencephalogram

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 71.05/P17

Topic: D.17. Voluntary Movements

Title: Dependence of lateralized readiness potential on the prior probability of target-movement in a Go/Nogo task

Authors: A. FUJIKAWA, Y. MATSUMOTO, R. TAMAMURA, Y. KAKIMOTO, *O. ARAKI;
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Abstract: Lateralized readiness potential (LRP), which is the potential difference between the left and right hemispheres in the motor related areas immediately before movement, is concerned with movement preparation and execution. Furthermore, LRP is considered to be affected by the prior information about the expected movement. However, it is unclear how the prior probability (PP) of cued information affects LRP in a reaching task, where the visual cue indicates left or right hand under the condition that the indicated hand should be moved finally. In a previous study, Miller (1998) reported contralateral LRP to a hand movement with 75% probability when left or right hand movement is cued with 75-25% PP. On the other hand, Scheibe et al. (2009) attempted three conditions of 100-0%, 75-25%, and 50-50% PPs and reported that LRP were not observed except 100-0%. These studies reported conflicting results as follows: LRP occurs when the PP is 100% only or when the PP is quite high. The issue that the LRP to 75-25% condition is

uncertain will be due to possible cancellation of readiness potentials in both hemispheres. Thus, lateralizing readiness potential to a hemisphere by indicating left or right hand and PP of the target action at the same time, we aim to reveal whether LRP occurs or not when the PP is not 100% and further the quantitative relationship between PP and LRP. We recorded electroencephalography of 13 subjects during a reaching task, where a spatial cue with an instruction which hand to use was presented at first and the subject was required to move the hand to the target or not, following the next Go or Nogo cue. Subjects were allowed to prepare for the action based on PP because the thickness of lines in the spatial cue implies go probability, i.e. one of 100%, 80%, and 20%. The result showed LRP occurrence when the PP is 80%. To reveal the effect of PP, we compared LRPs in different PP conditions for 100ms just before the Go/Nogo cue was presented. When PP was 100% and 80%, LRP were significantly observed, but were not when PP was 20%. In addition, the LRP amplitude with 100% PP is larger than that with 80%, and the LRP with 80% PP is larger than 20%. The average response times for 100% and 80% PPs are 482 ms and 521 ms, respectively. Both are shorter than 603 ms in the control condition ($p < 0.001$), that confirms the preparation for actions in these conditions. The results suggest the dependence of LRP on PP such that the higher the PP is, the larger the LRP amplitude is. It is also suggested that the possibility of a partial preparing state for movement in the motor related cortical areas, which was observed as the smaller LRP.

Disclosures: **A. Fujikawa:** None. **Y. Matsumoto:** None. **R. Tamamura:** None. **Y. Kakimoto:** None. **O. Araki:** None.

Poster

071. Cortical Planning and Execution: Electroencephalogram

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: D.17. Voluntary Movements

Support: NSERC

Title: Differential effects of continuous theta burst stimulation (cTBS) over left premotor cortex (PMC) and right prefrontal cortex (PFC) on modulating upper limb somatosensory input

Authors: ***M. J. BROWN**, W. R. STAINES;
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Abstract: Somatosensory evoked potentials (SEPs) recorded from cortical surface electroencephalography (EEG) after median nerve (MN) stimulation measure mixed afferent

somatosensory input. SEPs recorded maximally over frontal electrodes, such as frontal N30 and N60 peaks, represent somatosensory processing in non-primary motor areas compared to parietal SEPs (i.e. P50) that represent relay into somatosensory cortices. Several neural areas including the premotor cortex (PMC) and prefrontal cortex (PFC) have been associated with the preparation and planning of upper limb movements. However, it is currently unclear how PMC and PFC contribute to the selection of somatosensory input to inform upcoming movement. In the current study, two experiments examined SEP modulations after continuous theta burst stimulation (cTBS) to produce transient disruptions of left PMC (Experiment 1) and right PFC (Experiment 2). In both Experiment 1 (n=15) and Experiment 2 (n=16) pre-post experimental designs had participants receiving vibrotactile (VibT) stimuli to either their left index (D2) or pinky finger while also receiving MN stimulation time-locked relative to VibT onset during pre-stimulus (250 ms before VibT), early response selection (250 ms after VibT), late preparatory (750 ms after VibT) and execution (1250 ms after VibT) phases. SEPs were compared between passive stimulation in the No Task condition to Attend and Move condition, where participants attended to VibT stimuli to D2 and executed a pre-matched finger sequence with the right (contralateral) hand to specific VibT targets. The key findings of Experiment 1 revealed significant decreases in N30 and N60 peak amplitudes after cTBS to PMC. In contrast, the results of Experiment 2, also found significant decreased N60 peak amplitudes as well as trends for increased N30 and P50 peak amplitudes. A direct comparison of Experiment 1 and Experiment 2 confirmed differential modulation of N30 peak amplitudes after PMC (gated) compared to PFC (enhanced) cTBS. Collectively, these results support that both the left PMC and right PFC have modulatory roles on early somatosensory input into non-primary motor areas represented by frontal N30 and N60 SEPs. These results confirm that PMC and PFC are both part of a network that regulates somatosensory input for upper limb motor control.

Disclosures: **M.J. Brown:** None. **W.R. Staines:** None.

Poster

071. Cortical Planning and Execution: Electroencephalogram

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 71.07/P19

Topic: D.17. Voluntary Movements

Title: The influence of movement observation on movement execution: an EEG study on automatic imitation

Authors: *L. ZAPPAROLI[1,2,3], J. KILNER[2];
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Abstract: Automatic imitation is a robust stimulus-response compatibility effect in which the characteristics of movement stimuli (non relevant for the task) facilitate compatible actions and interfere with the incompatible ones. Previous studies have explained the effect the observed movement has on the timing of the executed action in terms of incompatible motor programs underlying the different observed and the executed actions. Despite many behavioural studies that have replicated this automatic imitation effect, very little is known about the neurophysiological mechansims that underlie it. The aim of this study was to explore the neurophysiological underpinnings of this effect using EEG, with particular attention to the role of beta oscillations. In particular, our goal was to test whether the automatic imitation is related to the compatible pre-activation of the motor system during action observation. We tested the hypothesis that modulations in beta power in the motor system driven by the observed action prior to the imperative cue could account for variance in the reaction time to the imperative cue. To this end, we recorded The EEG from 18 young right-handed healthy participants using a 128 electrode active EEG system whilst the participants performed an automatic imitation task. In the task subjects observed short movies of moving hands (non-relevant stimulus), followed by an imperative cue that guided their responses. Participants were instructed to response as quickly and as accurately as possible to the imperative cue. For the behavioural data (reaction times) analysis we performed repeated-measures ANOVA. In agreement with previous studies we found significantly shorter reaction times for trials in which the motor response was compatible with the observed movement. For the EEG data, after a preprocessing phase, we performed a time-frequency analysis and averaged over the beta frequency range (15-30 Hz); we then analysed the EEG data with a general linear model approach with the reaction times as covariate of interest. We found significant changes of beta oscillations prior to the imperative cue during action observation over the cortical areas of the motor system. Furthermore, these changes were significantly correlated with the subsequent reaction time to the imperative cue. Our results demonstrate an important role of the beta oscillations in the prediction of how action observation influences the execution of a successive movement. These findings could have an important role for further research on neurological diseases that are characterized by deficit of inhibition of automatic movements (e.g. Tourette Syndrome).

Disclosures: L. Zapparoli: None. **J. Kilner:** None.

Poster

071. Cortical Planning and Execution: Electroencephalogram

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 71.08/P20

Topic: D.17. Voluntary Movements

Support: UoC EG CONNECT

Title: Phase-difference analysis of EEG-data in movement related tasks reveals common underlying network of synchronous activity

Authors: *N. ROSJAT[1,2], S. POPOVYCH[1,2], B. WANG[2], L. LIU[1,2], T. TOTH[1], S. VISWANATHAN[2,3], C. GREFKES[2,3], G. R. FINK[2,3], S. DAUN-GRUHN[1,2];
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Abstract: The vast majority of motor actions, i.e. their preparation and execution, is the result of a complex interplay of various brain regions. Novel methods in computational neuroscience allow us to assess interregional interactions from time series acquired with *in vivo* techniques like electro-encephalography (EEG). These methods have helped provide first insights as to how brain areas assemble into functional networks depending on the motor task. However, our knowledge on the neural signals that encode individual movements and how they are changed during stroke is relatively poor. EEG data (64 channel system) were recorded continuously from 17 right-handed healthy participants (27±4 years, 10 female) during a simple motor task. The participants had to execute left or right index finger tapping movements that were triggered either by a visual cue or by voluntary choice. We used phase-difference analysis of the Hilbert phases of the Laplacian referenced EEG data to identify the coupled brain regions during the motor task. We analyzed the connectivity for electrodes lying above the premotor areas (PM: FC3, FC4), the supplementary motor areas (SMA: Cz, FCz) and the primary motor cortex (M1: C3, C4). Our analysis revealed an underlying coupling structure during the preparatory phase of the movement in the delta-theta-frequency band (2-7 Hz) that is common to all conditions irrespective of the hand performing and the cue triggering the movement. We hypothesize that this underlying network of synchronous activity makes the motor system more excitable so that transient cue signals are able to induce different kinds of movements during movement preparation.

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Poster

071. Cortical Planning and Execution: Electroencephalogram

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 71.09/P21

Topic: D.17. Voluntary Movements

Title: Network effects on individual movement representations: Evidence from an EEG study

Authors: *B. WANG[1], S. VISWANATHAN[2,1], R. ABDOLLAHI[1], N. ROSJAT[3,1], S. POPOVYCH[3,1], S. DAUN-GRUHN[3,1], C. GREFKES[2,1], G. FINK[2,1];
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Abstract: The specialized circuits of the motor system can be flexibly recruited by very different cognitive networks. For example, the motor-system can be recruited differently to execute movements in response to an external stimulus, or, to an intention without a stimulus. Even though the executed movement itself may be identical, it is unclear whether and how the recruiting cognitive networks can affect the neural representations of the movement. We hypothesized that such different recruiting networks may affect the movement representations and different motor tasks will share common neural representation of the motor output if the executed movements are identical. We collected 64-channel EEG data from 21 young healthy participants while they made index-finger movements with either their right or left hands in two different contexts. In the Cued-condition, participants responded to an arrow cue that pointed either to the right or to the left by pressing a button with the corresponding hand. In the Self-paced condition, participants produced freely chosen right or left hand button-presses at regular intervals without an external cue. Accelerometers measured the finger accelerations to estimate the movement performance: processing time (PT) from the stimulus to the movement onset, and movement time (MT) from movement onset to button press. We found the P300 amplitude in parietal (Pz) correlates with the stimulus-related PT, while there was no relationship with the MTs. Interestingly, we show that individual mean MT were highly correlated between visually cued and self-paced condition despite of the differences of recruiting network. And, we found the increased lateralized readiness potential (LRP) in motor cortex is associated with the faster MT for both conditions even though the pattern of brain activity are different between two conditions. In addition, we show the synchronous LRP of parietal cortex involved in self-paced movement, while the synchronous LRP of prefrontal cortex in visually-cued movement, which further suggested the different recruiting network of different motor tasks in term of LRP. Together, our findings provided the electrophysiological evidence to reveal the common underlying mechanism that is independent of tasks and the recruiting network to control the motor output for two actions under the control of different neural network.

Disclosures: **B. Wang:** A. Employment/Salary (full or part-time):; Forschungszentrum Juelich. **S. Viswanathan:** None. **R. Abdollahi:** None. **N. Rosjat:** None. **S. Popovych:** None. **S. Daun-Gruhn:** None. **C. Grefkes:** None. **G. Fink:** None.

Poster

071. Cortical Planning and Execution: Electroencephalogram

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 71.10/P22

Topic: D.17. Voluntary Movements

Support: UoC EG CONNECT

Title: Phase-locking in the delta-theta band is an EEG marker of movement execution

Authors: ***S. GRUHN**[1], S. POPOVYCH[2], N. ROSJAT[2], B. WANG[3], L. LIU[2], C. GREFKES[2], G. R. FINK[4];
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Abstract: Motor actions are the result of a complex interplay of various brain regions. Since the same brain regions can assemble into different functional networks depending on the action, identifying the neural signals that encode an action's component (such as selection, preparation and execution) remains a challenging task. In the study reported here, we sought to identify neural markers of a movement's execution that are invariant under differences in the functional networks in which movement is initiated. EEG data (64 channel system) were recorded continuously from 17 right-handed healthy participants (27±4 years, 10 female) as they performed a simple motor task. The task required participants to execute a left or right index finger tapping triggered by (i) a visual cue, or (ii) by an uncued voluntary choice. Despite the substantial differences in how the movements emerged in these two conditions, we hypothesized that the common movements executed in both conditions could be associated with common EEG markers. We used time-frequency and phase-locking analysis of the Laplacian referenced EEG data centered on movement onset. We analyzed the EEG signals recorded from the electrodes which were located above different regions of the motor cortex (C2 and C4 over rM1, C1 and C3 over lM1, FC3 over lPM, FC4 over rPM, FCz and Cz over SMA) and used the electrode Oz (above the visual cortex) to record and check the visual activity in the cued movement condition. Data analysis revealed that both the voluntary and the visually triggered movement condition exhibited the same significant phase-locking effect in the delta-theta frequency band (2-7 Hz) of the EEG signals at the electrodes above the contralateral motor regions, irrespective of whether

the left or the right index finger performed the movement. Our results suggest that phase-locking in the delta-theta frequency band is an electrophysiological marker of the execution of movement no matter how it is initiated.

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Poster

071. Cortical Planning and Execution: Electroencephalogram

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NSF ENG-1137279 (EFRI M3C)

ONR MURI Award No.: N00014-10-1-0072

Title: Delay differential analysis: a framework for multimodal non-linear classification of Parkinson's disease

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Abstract: Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder in the world, yet has no standard diagnostic test. PD is known to lead to marked alterations in cortico-thalamo-basal ganglia activity and subsequent movements, which may provide a biomarker for PD diagnosis. DDA is a time domain analysis framework based on embedding theory in non-linear dynamics. An embedding reveals the nonlinear invariant properties of an unknown dynamical system (here the brain) from a single time series (EEG or behavioral signals). The DDA embedding serves as a low-dimensional nonlinear functional basis onto which the data are mapped. The combination of behavioral and neurological observations gives rise to a multimodal analysis framework that will improve the understanding and classification of

neurological disease. We demonstrate how 750 ms of multimodal data can be used to improve DDA classification performance of PD after an unexpected perturbation of a virtual target during reach to grasp movements. We found that the anteroposterior hand position and hand aperture, in particular, provide improved classification performance in comparison to clean EEG data, as evaluated by the area under the ROC curve (AROC), (AROC increases from 0.71 to 0.81 with the addition of behavioral data). Thus, multimodal DDA may provide a tool for aiding the clinician in the diagnosis of PD and allow for earlier intervention with disease modifying therapeutics.

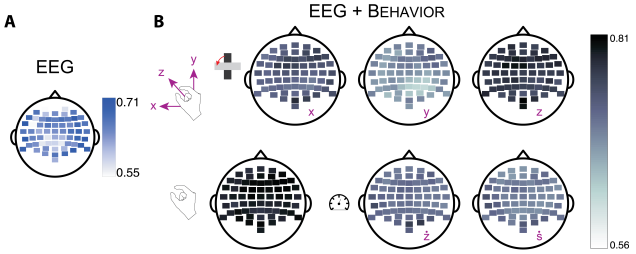

Figure 1. PD off-medication versus Controls classification performance of DDA of A) EEG data and EEG and behavioral data during reaching to grasp movements during virtual target perturbations, as evaluated by the area under the ROC curve (AROC).

Disclosures: M.E. Hernandez: None. **J. Weyhenmeyer:** None. **C. Lainscsek:** None. **T. Sejnowski:** None. **H. Poizner:** None.

Poster

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BBSRC David Phillips fellowship (UK)

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FWO Odysseus project (Fonds Wetenschappelijk Onderzoek, Belgium)

Title: The neurophysiological correlate of perceptual sensory attenuation measured using a force-matching paradigm

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Abstract: During movement incoming sensory information is filtered via central mechanisms: sensory gating. This gating has been described at two levels. At a neurophysiological level cortical electroencephalogram (EEG) recordings have highlighted a suppression of somatosensory evoked potentials (SEPs), produced in response to electrical stimulation of the median nerve, prior to and during movement onset. At a perceptual level, sensations which are self-generated are perceived as less intense than those which are externally generated, which explains why we are unable to tickle ourselves. A force matching paradigm in which participants must match a target force either by pushing on themselves or via an external mechanism is classically used to quantify this phenomenon. Participants consistently overestimate matched forces during the self-matching condition. It has previously been assumed that the sensory gating of SEPs during movement underlies this perceptual sensory attenuation, however specific predictions regarding this have not been addressed. The aim of this study is to identify the neurophysiological correlates of the percept of sensory attenuation. In this experiment 12 participants carried out a traditional force matching task whilst brain activity was recorded using EEG. Participants received median nerve stimulation 1) during phasic muscle contraction whilst finding the perceptually equal force (on select trials); 2) during isometric contraction when holding the matched force. Both right and left median nerves were stimulated in separate sessions. A control condition was included in which participants received a series of median nerve stimuli at rest (baseline) and whilst producing a self-paced movement of the index finger of the stimulated hand. Consistent with previous findings: we found that participants significantly overestimated the perceived force during the self-generated condition compared to the externally-generated condition. Interestingly, we found as expected a significant attenuation of the SEP secondary complex in the control movement condition compared to baseline, but no significant difference in SEP component amplitudes in the self-generated vs the externally-generated condition. This data suggests neurophysiological gating of SEPs in response to movement may occur via a different mechanism to the perceptual sensory attenuation observed in response to self-generated vs externally-generated movements. Further EEG analyses in the time-x-frequency domain will aim to uncover the neurophysiological correlate underlying this perceptual sensory attenuation.

Disclosures: C.E. Palmer: None. **M. Davare:** None. **J.M. Kilner:** None.

Poster

072. Cortical Planning and Execution: Neuroimaging

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Support: NSF BCS-1455629

NSF BCS-1341143

James S. McDonnell Foundation 220020293

Title: Shared patterns of activation during tool-use and tool-making in the human brain: an ALE meta-analysis

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Abstract: The manufacture and use of complex tools is one of the most distinctive human behaviors. Humans have a left lateralized tool-use circuit involving areas in the inferior frontal gyrus (Broca's area), dorsal and ventral premotor cortex (PMD, PMV; area 6), superior and inferior parietal lobes (SPL, IPL), and temporal lobe. Neuroimaging studies of human stone tool-making have found activation patterns resembling that of tool-use. We thus performed a quantitative activation likelihood estimation (ALE) meta-analysis (GingerAle 2.3) on results from published whole-brain studies to identify clusters activated across tool-use and stone tool-making. A total of 387 foci were selected from 32 published tool-use studies consisting of tool-use planning, imagery, pantomime, and execution. Tool-making foci (78) were selected from four tool-making experiments, consisting of novice, trained, and experienced tool-makers. We performed a cluster level analysis on each group (cluster level inference: $p \leq 0.05$, permutation threshold = 1000, cluster-forming threshold: uncorr. $p \leq 0.001$) and a contrast analysis identified shared and unique activity between groups (uncorr. $p \leq 0.001$, p value permutations = 10000, min. cluster volume = 250 mm^3). The meta-analysis confirmed a left lateralized tool-use circuit that overlapped with tool-making (ALE value = 0.01), with shared activation in the left PMD (MNI: -23, -7, 61; -28, -6, 58), Broca's area (-53, 5, 30), somatosensory cortex (-46, -32, 45), and intraparietal sulcus (-39, -40, 44). Tool-making showed activity not observed in tool-use studies (ALE value = 3.15-3.89), including bilateral activation in posterior SPL (-28, -64, 56; 25, -55, 58) and visual cortex (-15, -71, 7; -18, -86, 31; 19, -91, 23; 16, -78, 11; 28, -79, 6), and left activation in PMD (-21, 6, 55) and anterior IPL (-46, -37, 41). Significant tool-making clusters in the visual cortex were observed in novice tool-makers, while PMD, posterior SPL, and anterior IPL activity was not associated with tool-making experience. Tool-making shares much of its neural circuitry with tool-use but activates additional areas that may support increased visual demands during stone tool manipulation. Although tool-based processing likely drives much of

the shared activity, processing of sequenced information may also contribute to shared circuitry. Broca's area and IPL in particular are also involved in sequential aspects of speech and language such as word generation and syntax. Investigating shared circuitry between language and tool processing may reveal how more efficient sequential processing might have evolved to support these distinctive human characteristics.

Disclosures: L.D. Reyes: None. **S. Bianchi:** None. **C.C. Sherwood:** None.

Poster

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Topic: D.17. Voluntary Movements

Support: FISM project n. 2011/R/8

Title: Brain reorganization following upper limb motor rehabilitation in patients with multiple sclerosis

Authors: *L. BONZANO[1], A. TACCHINO[2], G. BRICHETTO[2], L. ROCCATAGLIATA[1], A. DESSYPRIS[1], P. FERACO[1], L. LOPES DE CARVALHO[2], M. BATTAGLIA[2], G. MANCARDI[1], M. BOVE[1];
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Abstract: Upper limb impairments can alter the ability to perform daily living activities in patients with Multiple Sclerosis (PwMS). Motor rehabilitation has the target to maintain the residual capacities to the highest possible level, but consistent data on its efficacy are lacking. Individual patients' outcomes are variable, due to variations in brain functional properties and to the adopted rehabilitation protocol. Brain adaptation occurs following damage in PwMS, and an efficient motor rehabilitation might influence this process. We investigated the effects of upper limb rehabilitation on motor function and related brain activity patterns in PwMS. Thirty PwMS in a stable phase of the disease were randomized to receive an active (AMT group, n=15) or passive motor rehabilitation treatment (PMT group, n=15). Both groups underwent twenty 1-hour treatment sessions, three times a week: the AMT group performed voluntary task-oriented exercises, whereas the PMT group was treated with passive mobilization of the different segments of both limbs. Before and after treatment, upper limb motor performance was evaluated by standard evaluation protocols and an engineered glove to quantify finger motor performance accuracy. In the same sessions, brain activity was investigated by fMRI at 1.5 T

during sequences of finger opposition movements with the right hand following a metronome at 2 Hz; thus, all patients were asked to perform exactly the same task in all sessions, allowing the comparison of brain activations between groups and sessions (performance during fMRI was recorded by an MR-compatible glove). Laterality Index (LI) was calculated in the frontal lobe (Brodmann Area - BA 4 and 6), parietal lobe (BA 2/3 and 40) and cerebellum. Motor performance improved in all PwMS as effect of treatment. At baseline, in both groups task-related activations were found bilaterally in the sensorimotor areas, and cerebellum. After treatment, activity was reduced in the right sensorimotor and supplementary motor areas ("compensatory" areas) in the AMT but not in the PMT group. Particularly, in the AMT group activations were strongly left lateralized in the cerebrum and right lateralized in the cerebellum; in the PMT group the right BA 6 and left cerebellum were also activated. Accordingly, LI indicated increased laterality in all brain regions in the AMT group, but only in the parietal lobe in the PMT group. The active motor treatment was able to reduce brain resource demand, altered by the disease, and should be preferred to passive mobilization: motor planning and execution in voluntary movements integrate proprioceptive information and can be important stimuli for neuroplasticity.

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Poster

072. Cortical Planning and Execution: Neuroimaging

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Topic: D.17. Voluntary Movements

Support: Huffines Institute for Sports Medicine and Human Performance

Title: Age-specific cortical control during quadriceps activation: a fNIRS investigation

Authors: ***E. MORA**[1], R. MEHTA[1], S. MAPAR[2];
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Abstract: Existing neuroimaging capabilities have limited hemodynamic brain activation investigations of neuromuscular activation to smaller upper extremity or lower extremity (ankle dorsiflexion) muscles. Quadriceps strength is an important predictor of falls in the elderly and

quadriceps precision force control is an essential function for precision gait and balance. The use of functional near infrared spectroscopy (fNIRS) enables cortical measurements during activation of larger muscles and during dynamic tasks. The goal of this study was to examine age-related changes in brain activation patterns during maximum voluntary contraction (MVC) and precision force control (FC) of the quadriceps using fNIRS. Fourteen younger (6 M, 8 F; 23.2 (4.1) years) and eight older (4 M, 4 F; 72.6 (5.7) years) adults performed two different static knee extension tasks (MVC and FC). In the MVC task, participants were instructed to push as hard as they can for a total of 3 trials with 20 seconds rest. During the FC task, participants controlled knee extension force at the target level (30% of MVC) for 10 seconds with 20 seconds rest for a total of 6 trials. fNIRS data was recorded at 50Hz from Fp1 and Fp2 regions in the prefrontal cortex (PFC) and bilaterally from the supplementary motor areas (Motor A) and the primary motor cortex (Motor B). A 2 (age) x 2 (task) x 6 (brain regions) mixed-effects model was employed to investigate the main and interaction effects of these factors on the 10-second average oxygenated hemodynamic (HbO) response levels. Both PFC regions exhibited higher HbO levels than the left Motor regions ($F(5,17)=5.8$, $P<0.0001$), and younger adults exhibited greater cortical activation than older adults ($F(1,20)=4.8$, $P=0.03$). Brain region had significant interactions with age ($F(5,17)=3.8$,$P=0.002$) and task ($F(5,17)=2.5$, $P=0.03$). A significant task, brain region, and age interaction was found ($F(5,17)=2.9$, $P=0.01$); younger adults exhibited higher HbO levels during the MVC task than the FC task in the right PFC region, however, no such differences were found in the older adults. These results indicate that increased right PFC activation during MVC of the quadriceps in younger adults plays a major role in MVC activation that is not observed in older individuals or during FC tasks. These findings may account for why older adults may have more difficulty recovering from falls. Future work includes detailed investigation of the relationship between brain hemodynamics and biomechanical outcomes of quadriceps activation.

Disclosures: **E. Mora:** None. **R. Mehta:** None. **S. Mapar:** None.

Poster

072. Cortical Planning and Execution: Neuroimaging

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Topic: D.17. Voluntary Movements

Title: Reliability of functional near-infrared spectroscopy during expert juggling

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Abstract: This functional near-infrared spectroscopy (fNIRS) study focuses on the reliability of the cortical hemodynamic responses during juggling balls by experts. Comparing EEG and fNIRS, the latter is less sensitive for movement artifact induced noise and potentially a suitable technique for measuring cortical hemodynamic changes during the execution of spacious motoric movement (Rooks et al., 2010). During the execution of small movements like finger tapping cortical hemodynamic changes over central motor areas showed a good reliability (Plichta et al., 2007). However, spacious motoric movements with higher intensities cause higher hemodynamic responses (Harada et al., 2009). To our knowledge nothing is known about the reliability of these responses during the execution of spacious motoric movements like juggling. We recorded hemodynamic responses from 15 juggling experts (26.3 ± 5.2 years), able to juggle at least five balls for a duration of 20 seconds, in premotor, primary motor, primary somatosensory areas and the area MT/V5 on both hemispheres (Piper et al., 2014) using a recently developed portable 16-channel fNIRS device (NIRSport 88, NIRX GmbH, Berlin). Attenuation changes of both wavelengths (850 nm and 760 nm) were transformed to concentration changes of oxygenated and deoxygenated hemoglobin (oxyHb and deoxyHb, respectively) using the modified Beer-Lambert approach (Kocsis et al., 2006). Each juggler performed eight repetitive blocks of 2-ball (left and right hand), 3-ball, and 5-ball juggling, respectively, for 20 seconds in a randomized order. The experiment included a longer break of 20 minutes where optodes were not removed. Test-retest was performed by intra-session reproducibility of measurements, which were assessed channel-wise by intra-class correlation coefficients (ICC(3,1)) according to the procedure proposed by Hopkins (2000). Channel-wise contrasts of test with retest (t-test for change in the mean) for hemodynamic responses (grand-averages) during juggling tasks only differed for oxy-Hb during 2-ball juggling left and right hand as well as 5-ball juggling. Hemodynamic responses were lower in the second session as compared to the first. Both variables, concentration changes of oxyHb (3-ball juggling: ICC(3,1) = .69 - .93, 2-ball juggling right hand: ICC(3,1) = .63 - .89, 2-ball juggling left hand: ICC(3,1) = .72 - .86, 5-ball juggling: ICC(3,1) = .82 - .94) and deoxyHb (3-ball juggling: ICC(3,1) = .86 - .97, 2-ball juggling right hand: ICC(3,1) = .81 - .95, 2-ball juggling left hand: ICC(3,1) = .78 - .97, 5-ball juggling: ICC(3,1) = .81 - .96) showed fair to good test-retest reliability. This is the case for all measured channels.

Disclosures: R. Wollny: None. **C. Andrä:** None. **M. Clauß:** None. **J. Mehnert:** None. **D. Carius:** None.

Poster

072. Cortical Planning and Execution: Neuroimaging

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Topic: D.17. Voluntary Movements

Support: JSPS KAKENHI 25.212

JSPS KAKENHI 15J03233

James McDonnell foundation

Title: Distributed representation of movement hierarchy in human neocortex

Authors: *A. YOKOI[1,2], U. HERTZ[1], E. BAMBER[3], J. DIEDRICHSEN[1];
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Abstract: The idea of motor chunking (Lashley, 1951) is that the brain breaks down longer sequences of movements into smaller sub-sequences, which enables superior memory and motor performance. While there have been a number of behavioural studies supporting the concept (Rosenbaum et al., 1983; Sakai et al., 2003), it is still unknown how motor chunks are hierarchically represented across cortical motor regions. To address this issue, we analysed the functional magnetic resonance imaging (fMRI) data from 12 human participants, which performed 8 different trained sequences in the scanner. We externally imposed a chunk structure through the way the sequences were memorized: On the first day of training, participants were taught 8 individual chunks of 2 or 3 presses. On the four subsequent days of training, they then learned to produce 8 sequences, each of which were composed of 4 of those chunks (11 presses total). Basic movement parameters were matched for all sequences. During scanning, participants repeated the same sequence twice within 8 seconds. The order of the 8 sequences was randomized and each sequence type was repeated three times in each of the 9 runs. Using representational similarity analysis (Kriegeskorte et al., 2008), we then tested whether the similarity structure of brain activity pattern follows the structures predicted by various representational models of sequence representation. A post-scanning behavioural test indicated that the imposed chunk structure in the early training significantly influenced the performance. The inter-press-intervals within a chunk were significantly faster than other intervals ($p<0.001$), both when chunks occurred within trained sequences or in the context of novel sequences. Representational fMRI analysis revealed that there was a regional-specific mixture of representations. Whereas the representation in contralateral M1 was mostly dictated by single finger presses, contralateral premotor and bilateral parietal areas showed clear evidence of representations for chunks and whole sequence in a spatially segregated manner. Furthermore, a non-hierarchical representation of transitions of finger presses was found in the ipsilateral dorsal

premotor cortex and the SMA. In summary, our results suggest that premotor areas represent learned sequences both in a hierarchical and non-hierarchical fashion.

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Poster

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Topic: D.17. Voluntary Movements

Support: FIRB 2013 (project RBFR132BKP)

Title: Interactions between different parieto-frontal cortical pathways during action execution

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Abstract: Our interactions with objects are strongly influenced by their physical properties. However, not only object's features can drive an action. Also our hand configuration, in terms of shape and orientation, can be easily modified to flexibly interact with them. The neural correlates subtending our capacity of reaching and grasping objects, i.e. prehension, have been classically localized within a fronto-parietal cerebral network. This system includes a dorsomedial pathway, considered to process reaching information, and a dorsolateral pathway, considered to process grasping information. However, recent studies have challenged this classical subdivision by showing coding for grasp-related information, such as grip type and hand orientation, within both pathways. Consequently, it is yet unclear if there is an exchange of information between these two pathways or if they independently code similar action properties. Here we adopted dynamic causal modelling (DCM) analysis on fMRI data to investigate the connectivity between and within the two pathways. Participants were requested to perform non-visually-guided grasping actions directed on an object. The actions were executed with their right hand while lying in the MR scanner. We used a 2x2 factorial design, including grip type (precision grip vs. whole hand prehension) and hand orientation (0° vs. 90°) as factors. We showed a modulatory effect of the considered experimental factors, grip type and hand orientation, on the connectivity profiles within the prehension network. DCM analysis provided evidence for an exchange of information between the two pathways, suggesting that the two dorsal streams are not functionally independent. Our study offers new insights into the connectivity dynamics occurring

within the prehension network, supporting the idea of inter-regional interactions based on specific properties of the performed action.

Disclosures: **G. Malfatti:** None. **A. Lingnau:** None. **L. Turella:** None.

Poster

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Support: Florida Biomedical Grant 3KN01

Title: Visual gain induced changes in visuomotor system activity in chronic stroke

Authors: ***D. B. ARCHER**, G. MISRA, C. PATTEN, S. MARBLE, S. COOMBES;
Univ. of Florida, Gainesville, FL

Abstract: The visuomotor network has been extensively studied in healthy individuals. Recent motor neuroimaging studies have associated region specific changes in the visuomotor network with changes in the properties of visual feedback such as visual gain. Increasing visual gain (i.e. magnifying error) during a visuomotor task reduces force error in chronic stroke individuals, but corresponding changes in functional activity in and beyond the visuomotor network have not been studied in this cohort. Previous visuomotor fMRI studies show that stroke individuals engage the visuomotor network more bilaterally, and that contralesional activity increases with task difficulty. Here, we used functional MRI to examine the functional brain activity that is associated with visual gain related improvements in force performance in chronic stroke individuals as compared to healthy age-matched controls. Chronic stroke and control individuals performed three visuomotor tasks during fMRI with their impaired/non-dominant hand. Visual gain was parametrically increased between tasks. The stroke group had greater error as compared to the control group at all gain levels, but both groups showed a progressive decrease in force error with an increase in visual gain. Increasing visual gain led to increased activity in key visuomotor regions such as the extrastriate visual cortex, premotor cortex, and inferior parietal lobule in stroke and control groups. Our observations also revealed increased activity in the contralesional premotor and primary motor cortex in the stroke group. Together, our findings show that visual-gain related improvements in force performance in chronic stroke are associated with a progressive increase in functional activity in the visuomotor network. We also found evidence of increased activity in ipsilateral motor areas in the stoke group across all levels of

visual gain. We conclude that changes in visual gain during a visuomotor task in chronic stroke lead to increased activity in regions within and beyond the visuomotor network.

Disclosures: D.B. Archer: None. **G. Misra:** None. **C. Patten:** None. **S. Marble:** None. **S. Coombes:** None.

Poster

072. Cortical Planning and Execution: Neuroimaging

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Topic: D.17. Voluntary Movements

Support: NIH Grant 5K12HD073945-02

Title: Precise hemodynamic control of primary motor cortical activity using an adaptive feedback paradigm

Authors: *E. OBLAK[1], M. U. JAKOB[2], J. S. SULZER[1];
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Abstract: Real-time fMRI (rtfMRI) gives us a whole-brain neural signal that can be used as an indication of an individual's current brain state. An exciting prospect of measuring neural states in real-time is to be able to guide individuals toward specific neural states, for example to enhance task performance or to facilitate neurorehabilitation. The most common approach thus far has been to use the brain state signal as feedback to the participant. More recent iterations have developed adaptive paradigms capable of triggering or changing a stimulus based on a real-time brain state, without the participant's knowledge of their own brain activity. Such paradigms may be advantageous as they can overcome confounding issues with the varying cognitive abilities of different individuals. Our approach expands these processes via an adaptive control system. This system calculates the difference between the current real-time brain state and the desired brain state, and then uses an a priori model to change the ongoing task to achieve a brain state that is closer to the desired one. Here, we apply this concept by developing a system to guide an isometric motor task in two stages: in the first fMRI session, we characterize the neural circuit involved in the task to develop the initial parameters for an adaptive controller; and in a second fMRI session, we apply an adaptive controller to fine-tune the model during task execution. Specifically, in the first session, we parameterize the BOLD activity in the hand area of the primary motor cortex (M1) of one subject based on force applied and the complexity of an isometric precision grip task; and in the second stage, we successfully apply this adaptive

controller to another subject in a subsequent fMRI session to achieve M1 BOLD percent signal changes (PSC) of 0.33, 0.66, and 1.00. Our preliminary results show that it is possible to use an rtfMRI signal to adaptively adjust a motor task to achieve a specific neural state. This work represents the first model-based hemodynamic control system with error-based reference tracking. Future iterations will extend this adaptive control technique to differential region of interest control as well as control of multi-voxel fMRI patterns within motor cortical structures.

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NCRR NCATS CTSA 8UL1TR000055

Title: Neural correlates of nonlinear integration of visual and proprioceptive feedback during wrist stabilization

Authors: *A. J. SUMINSKI[1], R. A. SCHEIDT[2];
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Abstract: Limb stabilization is a fundamental motor behavior requiring closed loop feedback control. The availability of multiple feedback sources (e.g. vision and proprioception) raises the question of how sensory information is combined to drive feedback compensation for kinematic performance errors caused by perturbations encountered during stabilization. Here we investigated how the neural mechanisms involved in feedback control of limb position were modulated in response to manipulation of the fidelity between visual and proprioceptive information. Twelve healthy subjects steadied their wrists while resting supine in a 1.5T MRI scanner. They stabilized against either predictable, constant extensor torque perturbations (CT) or unpredictable, pseudo-random extensor torques (RT) having the same average extensor torque as the CT perturbation. Direct view of the arm was precluded, although a cursor representing wrist angular deviations from a target was visible via prism glasses. One of 3 types of visual

feedback was available: true vision (TV), pseudo-random vision (RV) and no vision (NV). Scanning runs were made up of 30 s of stabilization in each of the 6 conditions, with 30 s of rest preceding and following each stabilization period. Behaviorally, performance errors accumulated more quickly during trials where the fidelity of visual feedback decreased. Analysis of functional images was conducted in 2 stages. First, a blocked design multilinear regression analysis revealed expected task-related activations in a cerebello-thalamo-coritcal circuit previously linked with feedback stabilization. Importantly, we found that BOLD activity within this network varied based on the fidelity of visual feedback. A second regression analysis, performed on residuals from the blocked analysis, evaluated correlation between BOLD signal fluctuations and kinematic performance errors during RTRV stabilization. Importantly, BOLD signals in areas supporting feedback stabilization only exhibited correlation with hand errors, not cursor errors. In comparing BOLD signal correlations with hand errors during the NV and TV conditions, we found that addition of veridical visual feedback caused marked changes in the overall network activity (a drop-out of prefrontal activation as well as dramatic increases in parietal and cerebellar areas engaged in feedback control). These results favor a nonlinear, competitive integration strategy when V and P are in conflict. This nonlinearity is further reflected in the observation that relative to the NV condition, addition of veridical visual information modifies the network structure mediating sensory feedback control.

Disclosures: A.J. Suminski: None. **R.A. Scheidt:** None.

Poster

072. Cortical Planning and Execution: Neuroimaging

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Topic: D.17. Voluntary Movements

Support: NEXT Program

Neuro Creative Lab

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Title: Comparison of decoders in multi-voxel pattern analysis for identifying task-specific resting-state brain activity in primary motor cortex

Authors: *T. KUSANO[1], H. KURASHIGE[3], I. NAMBU[2], Y. MORIGUCHI[4], T. HANAKAWA[5], Y. WADA[2], R. OSU[6];

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Abstract: The human brain activity at rest by functional magnetic resonance imaging is called resting-state fMRI (rs-fMRI), which has been shown its physiological significance for memory, maintenance of brain function, and creative thinking, and it is used for a biomarker for brain disease or classifying internal states. To classify brain states or identify fine brain activity patterns specific to a task (event), multi-voxel pattern analysis (MVPA) has become popular in fMRI analysis. However, MVPA applied to rs-fMRI has not been examined well. The purpose of this study is to investigate applicability of MVPA for rs-fMRI to identify fine brain activity patterns in the human motor cortex. In many cases, MVPA is usually applied to whole brain which includes large areas. Support vector machine (SVM) is commonly used as a decoder (classifier) because it generally shows high classification accuracy. However, we do not yet know that SVM is the best decoder for rs-fMRI. In this study, we compared two decoders: SVM and regularized logistic regression (RLR). We designed a decoder to detect voxel-level neural representations corresponding to task-specific activation in primary motor cortex (M1). Thus, each decoder was trained using the data in which the subject performed wrist/finger flexion movement. Next, we applied the decoder to rs-fMRI and defined obtained result as wrist versus finger index (WFI): how much rs-fMRI pattern is similar to each task-induced pattern. Finally, to confirm that resting-state brain activity reflects task-induced brain activity pattern, we compared WFI using weight parameter of designed decoder and that using random shuffled weight parameters by evaluating standard deviation (SD) of WFI distribution. Results showed that the average accuracy of each decoder was over 80% and RLR obtained higher accuracy than SVM. More than half of subjects showed significantly larger SD than that using random WFI histogram in each decoder, and the number of subjects showing significantly larger SD was larger in RLR. These results suggest that the resting-state brain activity includes multi-voxel patterns similar to the neural representation for the tasks and RLR is more adequate than SVM in MVPA for rs-fMRI.

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Poster

072. Cortical Planning and Execution: Neuroimaging

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Program#/Poster#: 72.11/P35

Topic: D.17. Voluntary Movements

Title: Getting a handle on Virtual Tools: An examination of the neuronal activity associated with virtual tool use

Authors: *A. RENO, K. FERCHO, R. FINN, T. BOSCH, L. A. BAUGH;
Basic Biomed. Sci., Univ. of South Dakota, Vermillion, SD

Abstract: Research has demonstrated that tool use is primarily supported by left hemisphere brain networks responsible for tool identification and tool action knowledge. With the advent of computers, virtual tools have become increasingly important in our day-to-day lives. Virtual tools can be thought of as those tools that have a separation between the tool's effector and the tool user. A common example is a computer mouse in which the onscreen movement of the cursor is not necessarily mapped in a one-to-one fashion with hand movements and lacks much of the somatosensory feedback that is common among real tool use. Recent behavioral work has provided evidence that these virtual tools may be represented in a similar manner to real tools by the brain. Using a virtual tool manipulated to hit targets appearing at various eccentricities during a functional magnetic resonance imaging (fMRI) task, this study assessed whether virtual tools activate the same neuronal networks elicited by real tool use. Participants performed in a single fMRI experiment consisting of two experimental conditions, each with a reversal and non-reversal block of trials. In the first condition (Vision), a visual schemata illustrating the relationship between hand and cursor position was provided. During the reversal trials, this information consisted of a stick pivoting around a center point connecting hand position and cursor position; therefore, leftward hand movements were required to hit targets appearing on the right. During the non-reversal trials, the relationship between hand and cursor was represented by a stick connecting hand position and cursor that translated with hand movement. The second experimental condition (No Vision) was similar to the first, but the stick was not made visible to participants. Subject's hand movements were sampled throughout the experiment while functional neuroimaging was performed. Behavioral results matched previously reported reaction time effects, with vision of the tool in the reversal condition decreasing movement initiation times when compared to conditions in which vision of the tool was not provided. Vision of the tool elicited activity within the left hemisphere ventro-dorsal and dorso-dorsal visual streams, regions previously identified as being critical in tool use. Activity within the posterior middle temporal gyrus, superior supramarginal gyrus, anterior intraparietal cortex, dorsal premotor cortex, and supplemental motor area all showed condition dependent activity. The present study demonstrates that the brain capitalizes on a similar network of regions to support the use of virtual tools as those utilized during real-world tool use.

Disclosures: **A. Reno:** None. **K. Fercho:** None. **R. Finn:** None. **T. Bosch:** None. **L.A. Baugh:** None.

Poster

072. Cortical Planning and Execution: Neuroimaging

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Program#/Poster#: 72.12/P36

Topic: D.17. Voluntary Movements

Title: Cortical activity during expert juggling

Authors: *D. CARIUS[1], C. ANDRÄ[2], M. CLAUß[2], J. MEHNERT[3], M. BUNK[4], R. WOLLNY[1];
[1]Martin Luther Univ. Halle-Wittenberg, Leipzig, Germany; [2]Univ. of Leipzig, Leipzig, Germany; [3]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [4]Inst. of Applied Athletics Training, Leipzig, Germany

Abstract: This functional near-infrared spectroscopy (fNIRS) study focuses on cortical hemodynamic changes during juggling balls by experts. Although current findings suggest structural changes by long lasting training (Gerber et al., 2014; Scholz et al., 2009), little is known about the functional changes during the execution of spacious motoric movements and their dependence on the expertise of the juggler. We recorded hemodynamic responses from 15 juggling experts (26.3 ± 5.2 years), able to juggle at least five balls for a duration of 20 seconds, in premotor, primary motor, primary somatosensory areas and the area MT/V5 on both hemispheres (Hashimoto et al., 2006; Mehnert et al., 2013, Piper et al., 2014) using a recently developed portable fNIRS device (NIRSport 88, NIRX GmbH, Berlin, Germany) with 8 light source and 8 detectors, which form 16 actual measurement channels. Attenuation changes of both wavelengths (850 nm and 760 nm) were transformed to concentration changes of oxygenated and deoxygenated hemoglobin (oxyHb and deoxyHb, respectively) using the modified Beer-Lambert approach (Kocsis et al., 2006). Each juggler performed eight repetitive blocks of 2-ball (left and right hand), 3-ball, and 5-ball juggling for 20 seconds in a randomized order. The 5-ball juggling expertise of the jugglers was quantitatively rated using a semi-automated cinematographic analysis (kinematic parameters: tosses per second, velocity of the toss, angle of the toss, both, for left and right hand). Due to our results, brain activity in the somato-motoric brain regions rises according to the subjectively rated level of difficulty across conditions (3-ball juggling < 2-ball juggling [in one hand] < 5-ball juggling). As hypothesized 5-ball juggling, by far, recruited most neuronal activity as compared to the other conditions in most of the channels. Furthermore, the level of expertise, acquired by cinematographic video analysis, is related to the functional changes of 5-ball juggling in the right primary motor area (R-M1) such that a higher level of expertise corresponds with less cortical activity (Spearmann's rank correlation; oxy-Hb: R-M1 $\rho = .694$, $p = .012$; deoxy-Hb: R-M1 $\rho = .718$, $p = .009$). The current study reports, according to our knowledge, for the first time transient cortical activity during the

execution of a spacious and complex motoric task: ball juggling and thereby provides the basis to research the interplay between functional and structural changes in the human brain induced by learning. Subjectively rated difficulty levels of the different conditions are associated with the amount of activity in the somato-motoric system.

Disclosures: **D. Carius:** None. **C. Andrä:** None. **M. Clauß:** None. **J. Mehnert:** None. **M. Bunk:** None. **R. Wollny:** None.

Poster

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Topic: D.17. Voluntary Movements

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USC Division of Biokinesiology and Physical Therapy

Loma Linda University Physical Therapy Department

Title: Brain connectivity associated with muscle synergies in humans

Authors: M. RANA[1], M. S. YANI[1], S. ASAVASOPON[2], B. E. FISHER[1], *J. J. KUTCH[1]; [1]USC, Los Angeles, CA; [2]Loma Linda Univ., Loma Linda, CA

Abstract: The human brain is believed to simplify the control of the large number of muscles in the body by controlling muscles in groups, termed muscle synergies. Muscle synergies are considered to be structured in spinal cord as modules of motor action, whose combination allows for a variety of movements and postures. However, the neural connectivity allowing the human brain to access and coordinate muscle synergies remains unknown. Here, we use a surprising pair of synergists in humans, the flexor hallucis longus (FHL, a toe flexor) and the anal sphincter, as a model well-suited to elucidate the neural connectivity underlying muscle synergy control. First, using electromyographic recordings, we demonstrate that voluntary FHL contraction is associated with synergistic anal sphincter contraction, but voluntary anal sphincter contraction occurs without FHL contraction. Second, using functional magnetic resonance imaging (fMRI), we show that two important medial wall motor cortical regions emerge related to these tasks - one located more posteriorly that preferentially activates during voluntary FHL contraction and one located more anteriorly that activates during voluntary FHL contraction and voluntary anal sphincter contraction. Third, using transcranial magnetic stimulation, we demonstrate that the

anterior region is more likely to generate anal sphincter contraction than FHL contraction. Finally, in a repository fMRI dataset of 48 men (www.nitrc.org), we estimated the functional connectivity of the anterior region and posterior region. We found that the anterior region has preferential connectivity to regions involved in the brain-bladder control network, including insula and dorsal anterior cingulate cortex. Whereas, the posterior region has preferentially connectivity to the regions involved in navigation networks, including primary somatosensory cortex, frontal cortex, parietal cortex, posterior cingulate and occipital cortex. We conclude that specific motor cortical regions in humans provide access to different muscle synergies, allowing distinct brain networks to coordinate the muscle synergy activation necessary for functional tasks. Thus, the interaction of brain networks encoding task demands with muscle synergy input nodes in the motor cortex may form the basis for the flexible combination of muscle synergies

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Poster

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Topic: D.17. Voluntary Movements

Support: NIDCD R01 DC012502

Title: Sensorimotor plasticity following speech motor adaptation

Authors: ***M. DARAINY**[1], S. VAHDAT[2], D. J. OSTRY[1];
[1]McGill Univ., Montreal, QC, Canada; [2]Univ. of montreal, Montreal, QC, Canada

Abstract: In recent publications there is increasing evidence showing that there are changes to sensory systems following motor learning and there are likewise changes to the motor system after perceptual training. It is fair to say, we may not have pure motor or somatosensory learning but rather sensorimotor learning. Here we have investigated the neural bases of speech motor adaptation. Subjects tested for two experimental sessions over two days. We used custom build dental appliances to connect subject's lower jaw to the robotic manipulandum and to stabilize the head. On the first day of experiment subjects were asked to repeat words shown on a computer monitor while the robot recorded their jaw movement without force application. Every 1.5 s a word chosen randomly from four words ("head", "said", "ted", "bed") came up on the screen and the subject was required to repeat it aloud as soon as possible. Afterward, subjects underwent

fMRI scans of the brain at rest. Then they returned to the lab to complete a perceptual discrimination task. In the perceptual discrimination task, two words “hid” and “head” or “head” and “had” were highlighted on the screen and then subjects heard a word, which was taken from a continuum that spanned the perceptual distinction between the highlighted words. They were required to classify the auditory stimulus as one of the words. On the second day of the study the order of procedures was exactly the same with one exception. Specifically, as the subject produced repetitions of the words, the robotic manipulandum applied forces in protrusion direction that increased in magnitude with vertical jaw displacement. Our behavioral data show that subject compensated for the action of robotic device. Moreover we observed consistent changes to perceptual boundary. We calculated behavioral indices of motor and perceptual change associated with learning and used them as predictor in the fMRI analysis. We found persistent changes in resting-state functional connectivity between auditory areas and both motor and somatosensory areas of the brain. The functional connectivity change in some of these connections correlated more with the motor learning while others correlated more with the perceptual change. Specifically, a network involving primary and secondary somatosensory cortex, primary motor cortex, cerebellar cortex and primary auditory cortex show changes in connectivity linked to motor indices of learning, while a network comprised of ventral premotor cortex, secondary somatosensory cortex, supplementary motor area, primary auditory cortex and inferior parietal lobule correlated more with the perceptual changes that occur in conjunction with learning.

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Poster

072. Cortical Planning and Execution: Neuroimaging

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 72.15/P39

Topic: D.17. Voluntary Movements

Title: Learning the exotic: Effects of direct experience with unfamiliar tools on motor resonance during tool use action observation

Authors: ***T. J. BOSCH**, K. FERCHO, A. RENO, L. A. BAUGH;
Basic Biomed. Sci., Univ. of South Dakota, Vermillion, SD

Abstract: When examining non-human primate tool use, research has shown that once trained to use a tool, populations of neurons within premotor cortex (F5/PM) and the inferior parietal lobule (PFG/ IPL) exhibit similar responses when both executing a tool use movement and

observing the same tool use, a process termed motor resonance. Tool use in humans elicits a similar resonance response within PM, IPL, and primary motor cortex (M1). An important question is whether gaining experience with a tool is necessary for motor resonance, as is observed in non-human primate research. In humans, differences have been noted between individuals with extensive experience with a tool and those with minimal experience, leading to the equating of motor resonance with action understanding. Whether differences in motor resonance result directly from experience or if those differences are present prior to obtaining experience is unknown. To directly assess this question, participants were trained to skillfully use chopsticks, a tool which many lack experience using. It was predicted that motor resonance activity would increase as experience with the tool was acquired. Participants underwent a baseline functional magnetic resonance imaging (fMRI) session in which a video of skilled chopstick use was presented. Following, four weeks of training was performed utilizing chopsticks to accomplish a skilled motor task. During training, participants picked up a marble from the bottom of a cylinder and dropped it into the top of the cylinder, repeating this action as quickly as possible. Behavioral measurements of task performance were recorded and eye movements were tracked to verify skill acquisition. Following training, a second fMRI scan was performed using an identical paradigm as the baseline scan and the neural activity obtained pre- and post-training was compared. Both behavioral and eye-tracking data confirmed all participants significantly improved in the use of chopsticks over the training period. When comparing baseline fMRI activity to post-training activity, significant differences within regions previously identified as exhibiting motor resonance (M1 and PM) were observed. This data confirm the hypothesis that motor resonance, as it is related to tool use, is dependent on extensive experience similar to non-human primates. These findings suggest that although humans are unique in their extensive repertoire of tool use capabilities, there is little evidence for a biological system supporting this ability, but rather that the motor resonance observed when using a variety of tools is likely the result of humanity's comprehensive experience with a variety of tools.

Disclosures: **T.J. Bosch:** None. **K. Fercho:** None. **A. Reno:** None. **L.A. Baugh:** None.

Poster

072. Cortical Planning and Execution: Neuroimaging

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 72.16/P40

Topic: D.17. Voluntary Movements

Title: Virtually perfect recall: An examination of the memory systems involved in the recall of complex virtual tool use

Authors: *K. A. FERCHO, A. RENO, T. BOSCH, L. A. BAUGH;
Basic Biomed. Sci., Univ. of South Dakota, Vermillion, SD

Abstract: Tool use relies on three visual streams (the ventral visual stream, ventro-dorsal visual stream, and dorso-dorsal visual stream), as well as the frontal lobe to understand, plan, and execute tool movement. An essential component of tool use is the ability to recall tool mechanics to guide the interaction between the body and the tool. Using a virtual tool manipulated to hit targets appearing at various eccentricities during a functional magnetic resonance imaging (fMRI) task, this study explored the neural activity underlying the recall of virtual tool dynamics. Two groups of subjects participated in a single fMRI experiment consisting of two experimental conditions, each with a reversal and non-reversal block of trials. In the first condition (Vision), a visual schemata illustrating the relationship between hand and cursor position was provided. During the reversal trials, this information consisted of a stick pivoting around a center point connecting hand position and cursor position; therefore, leftward hand movements were required to hit targets appearing on the right. During the non-reversal trials, the relationship between hand and cursor was represented by a stick connecting hand position and cursor that translated with hand movement. The second experimental condition (No Vision) was identical to the first, but the stick was not made visible to participants. The first group of subjects completed the Vision condition last (Group 1); the second group of subjects performed the Vision condition first (Group 2). To monitor behavioral performance, subjects' hand movements were sampled throughout each trial. By comparing the activity elicited in the reversal conditions of Group 1 and Group 2, an examination of the neural correlates associated with recalling virtual tool dynamics was possible. When participants were using the virtual tool in the reversal condition, areas of neuronal activity consistent with tool specific declarative and working memory were observed when contrasting Group 1 and Group 2. Activity included areas of the ventral visual stream, the intraparietal sulcus, and the superior parietal lobule. During the non-reversal conditions, no evidence of memory recall was found. Behavioral data supported the neuroimaging results with recall related activity related to increased performance. The data suggests that the simpler non-reversal condition of virtual tool use did not require the same memory systems and assumed recall of tool dynamics as was utilized for the reversal condition. This study offers an examination of the brain regions involved in the recall of information required to utilize a complicated virtual tool.

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Poster

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Topic: D.17. Voluntary Movements

Support: NIH/NINDS NS053962

Title: Persistent left hemisphere asymmetries in the structure of lower sensory and motor pathways in chronic right hand amputees

Authors: *H. PENG, C. M. CIRSTEA, S. H. FREY;
Psychological Sci., Univ. of Missouri-Columbia, Columbia, MO

Abstract: Background: Work in animal models indicates that deafferenting injuries precipitate experience-dependent structural changes at various levels of the central nervous system including major efferent (corticospinal, CST) and afferent (medial lemniscus, ML) tracts. We employ diffusion tensor imaging (DTI) and probabilistic tractography to identify changes in the CST and ML pathways in upper extremity amputees. Specifically, we ask whether the left hemisphere asymmetry exhibited by right-handers in tract structural organization is diminished following loss of the dominant right hand. **Methods:** DTI was collected in 19 (42% female, 48.2±13.1yrs) chronic (15.2±13.9yrs) traumatic right-hand amputees and 32 age-, sex-matched (38% female, 41.8±13.2yrs) healthy controls. All participants were right-hand dominant. We delineated the CST in each hemisphere by selecting a seed in cerebral peduncle with internal capsule waypoint and cortical terminus in Brodmann area 4 (based on the Jeulich Atlas). For the ML, the seed was placed in the brainstem with a waypoint in the thalamus and terminus in the Brodmann areas 1, 2, and 3. Fractional anisotropy (FA), mean diffusivity (MD), and tract volume were quantified separately for the 4 pathways' lower segments (between seeds and waypoints). DTI measures are reflective of myelin sheath integrity, axon diameter, axon density, and fiber organization. Inter-hemispheric asymmetries were quantified with a laterality index: LI=(Left−Right)*100/(Left+Right). **Results:** Controls displayed higher FA and lower MD for the left CST compared to the right CST ($p<0.001$ for both FA and MD), lower FA and lower MD for the left ML ($p<0.001$), but no differences in tracts volume ($p>0.05$). Amputees also showed no asymmetries in tracts volume ($p>0.05$). Despite chronic right hand absence, the amputees (like controls) showed higher FA and lower MD for the left CST ($p<0.001$) and lower FA ($p=0.03$) and lower MD ($p=0.005$) for the left ML. Ipsilateral ML FA correlated with time post-amputation ($r=0.52$, $p=0.02$). Based on LI, left hemisphere CST asymmetry was significantly larger in amputees than controls ($p=0.03$). **Discussion:** Our results highlight similar left hemisphere CST and ML asymmetry in both controls and amputees. In amputees, the motor reorganization at the brainstem and internal capsule levels may account for larger left hemisphere CST asymmetry. The absence of changes in tracts volume is consistent with the

preservation of both inputs and outputs. Positive relationship between ipsilateral ML FA and chronicity may indicate increased use of the intact hand (as shown in previous motor practice studies, Sampaio-Baptista et al., 2013).

Disclosures: H. Peng: None. **C.M. Cirstea:** None. **S.H. Frey:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.01/P42

Topic: E.01. Neuroendocrine Processes

Support: CNPq

Title: Hydrogen sulfide plays a propyretic role during endotoxic shock

Authors: *R. RESTREPO FERNÁNDEZ[1], R. SORIANO[1], H. D.C. FRANCESCATO[2], J. SABINO[2], T. MACHADO COIMBRA[2], L. BRANCO[2];
[1]Physiol., Univ. De São Paulo, Ribeirao Preto, Brazil; [2]Physiol., Univ. De São Paulo, Ribeirão Preto, Brazil

Abstract: Thermoregulatory modulators are known to increase (propyretic) or decrease (antipyretic) the febrile response to lipopolysaccharide (LPS). Endogenous hydrogen sulfide (H2S) is produced in the preoptic area (POA) mainly by the enzyme cystathionine β-synthase (CBS). At low doses of LPS POA H2S levels are reduced and this molecule is a powerful antipyretic acting through suppression of prostaglandin E2 synthesis. Conversely nothing is known about the effects of doses of LPS high enough to cause endotoxic shock and both hypothermia and fever. Therefore, the aim of this study was to test the hypothesis that POA H2S production is increased during hypothermia and decreased during fever in response to high doses of LPS, and that these alterations correlate with the observed changes in body temperature (Tb). Adult male Wistar rats were anesthetized and submitted to median laparotomy so as to insert a temperature datalogger capsule (SubCue, Calgary, AB, Canada) into the peritoneal cavity to record deep Tb. After 7 days rats received intraperitoneal injection (ip) of saline (1 ml/kg, n=9) or (LPS 2.5 mg/kg, n=18). Tb was recorded (5-min intervals), starting 1 h before treatments and ambient temperature was set at 25°C. Rats were decapitated 85 minutes (hypothermia; n=9) and 180 minutes (fever; n=9) after ip injection and bilateral samples of the POA were excised with a punch needle for measurements of H2S levels. LPS caused typical hypothermia and fever. Levels of POA H2S in euthermic rats (ip saline) were 0.9575 ± 0.1925 μg/mg protein/h. H2S

levels were significantly decreased during hypothermia (0.6756 ± 0.2342 μg/mg protein/h, n=9; P< 0.0134) when compared to fever (1.105 ± 0.2986). We also investigated the putative effect of increased central bioavailability of H2S on Tb. After median laparotomy anesthetized rats were implanted with a guide cannula in the third ventricle for intracerebroventricular (icv) microinjection. Ip injection of saline (n=21) or LPS (n=22) was followed by icv administration of a donor of H2S (sodium sulfide, Na2S; 4 nmol/1 μl, n=13) or saline (1 μl, n=9). Tb was recorded. Na2S did not affect Tb control during euthermia (ip saline). Endotoxic rats and icv microinjected with Na2S had a significant increased Tb during hypothermia phase (289.6 ± 11.31 °C × min; P< 0.0104.) when compared to saline icv (242.1 ± 12.37 °C × min). We conclude that the gaseous messenger H2S modulates hypothermia and fever during endotoxic shock induced by high doses of LPS, acting as a propyretic molecule.

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Poster

073. Neuroendocrine Anatomy and Physiology

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Program#/Poster#: 73.02/Q1

Topic: E.01. Neuroendocrine Processes

Support: CONACYT Grant 251509.

Title: NGF-induced PC12 cell differentiation and survival is inhibited by vasoinhibins

Authors: *Z. MELO, B. MORENO-CARRANZA, R. M. AROÑA, C. CLAPP, G. MARTINEZ DE LA ESCALERA;
Inst. de Neurobiologia, Univ. Nacional Autonoma de Mexico, Queretaro, Mexico

Abstract: Nerve growth factor (NGF) induces growth, differentiation and survival of peripheral neurons during embryonic development. Vasoinhibins (Vi) are a family of peptides derived from prolactin (PRL) that inhibit vasodilation and angiogenesis, functionally antagonizing the effects of various growth factors, including vascular endothelial growth factor (VEGF). In contrast to the abundant knowledge on the actions of Vi on endothelial cells, the information about the role played by Vi on the nervous system is limited. Vi have been detected in the SNC and are known to stimulate the release of vasopressin and to participate in the acute response to stress, increasing depression and anxiety-related behaviors. The aim of the present study was to evaluate the putative antagonistic action of Vi on the neurotrophic effect of NGF by measuring

survival and cell differentiation of PC12 pheochromocytoma cells. Our results show that the NGF-dependent neurite outgrowth is inhibited in the presence of human recombinant Vi. Vi (10 nM) reduced both the length and the density of NGF-induced neurites. PC12 cells infected with lentivirus carring a Vi gene were also resistant to the action of NGF (2 nM). Vi also reduced the metabolic activity, as determined by MTT assays, and increased apoptosis, as determined by DNA fragmentation measured by ELISA and TUNEL tests, in NGF-treated PC12 cells and NGF-treated Vi-expressing PC12 cells. These findings suggest that Vi perform a functionally antagonistic action over the effects triggered by NGF on periferal neurons, resulting in the abrogation of cell processes such as neurite outgrowth and survival.

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Poster

073. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

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CONACyT: 156413

Title: Artificial rearing condition affects the prolactin and growth hormone secretion in a primary culture of rat anterior pituitary cells

Authors: *C. G. TORIZ[1], C. SOLANO AGAMA[2], E. L. AGUIRRE-BENITEZ[3], A. MARTÍNEZ[5], I. JIMÉNEZ ESTRADA[2], J. HERNÁNDEZ FALCÓN[4], A. I. MELO SALAZAR[5], M. GONZÁLEZ DEL PLIEGO[3], M. E. MENDOZA GARRIDO[2]; [1]CINVESTAV-IPN, Mexico City, Mexico; [2]Dept. of Physiol. Biophysics and Neurosciences, CINVESTAV, México city, Mexico; [3]Dept. of Embryology, Fac. of Med., [4]Dept. of Physiol., Fac. of Med., UNAM, México city, Mexico; [5]CIRA, CINVESTAV-lab Tlaxcala, Tlaxcala, Mexico

Abstract: It is known (in rodents) that the communication between the hypothalamus and the pituitary is settling during the first two weeks of life, and any experimental manipulation or insult could affect the pituitary functionality. For example, Dwarf mice lacking somatotrophs

(growth hormones-secreting cell; GH) and lactotrophs (prolactin-secreting cells; PRL) exhibit a decrease in the number of tuberoinfundibular dopaminergic (TIDA) neurons, and low plasma levels of PRL. Exogenous administrations of PRL or GH prevent these effects. Furthermore, it is know that early in life there is an increment of PRL-cells and that bioactive compounds present in maternal milk during early lactation modified the number of these cells. It is believed that lactothophs originate from the somatotrophs through an intermediate phenotype cells known as somatolactotrophs (release GH and PRL). In pups that were separated from their mother and reared artificially (AR) we founded an increase in the serum levels of GH secretion at day 7 and a decrease of PRL secretion at 14 postnatal days (pnd). Also, we found an increase in the content of pituitary dopamine, without differences in the number of TH-positive hypothalamic neurons with regard to maternal rearing (MR) pups. Due to the above and because of lactotrophs secrete high levels of PRL in the absence of any hormone; we evaluated by a primary culture of rat anterior pituitary cells, whether there were differences in basal secretion. We found that there is an increase in the basal GH secretion at day 7 and a decrease in basal PRL secretion at day 14 in AR pups. These results are consistent with the previously observed at serum level. We also determined, by immunocytochemistry, the proportion of lactotrophs and somatotrophs cells in MR and AR pups of 7, 14 and 21 pnd. We did not find differences in the proportion of classic lactotrophs or somatotropes in all ages. We found a decrease in the proportion of somatolactotrophs cells (PRL/GH) at pnd 14, and an increase in the proportion of PRL-positive cells (PRL plus GH/PRL cells) at pnd 21. These results support the idea of importance of maternal milk in the process of maturation of the hypothalamic-pituitary axis.

Disclosures: **C.G. Toriz:** None. **C. Solano Agama:** None. **E.L. Aguirre-Benitez:** None. **A. Martínez:** None. **I. Jiménez Estrada:** None. **J. Hernández Falcón:** None. **A.I. Melo Salazar:** None. **M. González del Pliego:** None. **M.E. Mendoza Garrido:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.04/Q3

Topic: E.01. Neuroendocrine Processes

Title: The spatial relationships between endocrine cells in the anterior pituitary gland and blood vessels

Authors: M. YOSHITOMI[1], K. OHTA[2], A. TOGO[2], K.-I. NAKAMURA[2], *M. MORIOKA[1]; [1]Kurume Med. Sch., Kurume/ Fukuoka, Japan; [2]Div. of Microscopic and Developmental Anat, Kurume Univ. Sch. of Med., Kurume, Japan

Abstract: The relationships between the endocrine cells and the endothelial cells in anterior pituitary gland are important for hormone releasing. Endocrine cells and endothelial cells in the anterior pituitary gland frequently make contact between them. Such close relationship between them in the anterior pituitary gland is generally considered to facilitate its efficient secretory functions. However, numerical and spatial relationships between them are still unclear. To understand the whole relationship between them, we obtained the compleat three-dimensional cellural structures of anterior pituitary tissue with electron microscopic resolution by scanning electron microscope (SEM) based 3D reconstruction method using focused ion-beam SEM machinary (FIB-SEM) and we first demonstrated that about 70% of endocrice cell have contact sites to the endothelial cells but almost 30% of endocrine cells are existed in the tissue completely isolated from the perivascular space. Our 3D analysis visualize the detail localization of secretory granules (SG) in endocrine cells. The reconstruction data showed that an accumulation of SG in the region near by the contact site to the blood vessel. However, the SG in the cell which isolated from the perivascular region seems to be distributed uniformly in the cytoplasm. There is a significant difference between the cells which have contact to the vessel or not when we compared the vector quantity from center of gravities of whole cell to the SG region. These data suggested that the cellular interaction between the endcrine cell and encdothrerial cell promote the uneven distribution of the SGs within the cytoplasm and there is a possibility of the novel interaction between them.

Disclosures: M. Yoshitomi: None. **K. Ohta:** None. **A. Togo:** None. **K. Nakamura:** None. **M. Morioka:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.05/Q4

Topic: E.01. Neuroendocrine Processes

Support: KBRIN (NIGMS grant #P20GM103436) IDEA award to CC

Cedarlane Labs (in-kind)

Title: Neuroendocrine characterization of cell lines derived from adult female mouse hypothalamus

Authors: S. F. BAMJI[1], B. N. RADDE[2], P. MULUHNGWI[2], C. M. KLINGE[2], *C. CORBITT[3]; [1]Biol., [2]Dept. of Biochem. & Mol. Genet., [3]Univ. Louisville, Louisville, KY

Abstract: Immortalized neural cell lines derived from adult female mouse hypothalamus have recently become commercially available (Cedarlane Labs), but their phenotypes are only partially characterized. Five of these hypothalamic cell lines (mHypoA-50, 51, 55, 59 & 63) appear to have an arcuate nucleus phenotype based on initial transcript screening done by others, *e.g*., all express estrogen receptors (*Esr1* and *Esr2*) and some also express kisspeptin, leptin receptor, and/or neuropeptide Y. Our goal is to develop a model to determine the effects of glyceollin (Gly) in the hypothalamus. Gly is a soy product known to have both estrogen receptor (ER) -mediated (mainly anti-estrogenic) and non-ER mediated effects both *in vivo* and *in vitro* (primarily studied in cancer cells). We reported that i.p. administration of Gly affects expression of over 250 gene targets in female mouse brain. Several Gly targets (*Esr1*, *Esr2, Gper1, Nr4a1, Gh*, *Prl* and *Kiss1*) are made in arcuate nucleus. Using real-time qPCR, we report the first 4 targets are expressed by all 5 mHypoA cell lines in both basal and estrogen-stimulated conditions. We detected *Gh* in all cell lines, *Prl* in mHypoA-50, 51 & 59 and *Kiss1* in mHypoA-55, 59 & 63, although transcript levels were low compared to *Esr1, Esr2, Gper1*, and *Nr4a1*. Based on consistency of target detection and estrogen responsiveness, we conclude that mHypoA-55 & 63 cell lines are suitable for studying effects of Gly on both ER- and non ER-mediated pathways in hypothalamus.

Disclosures: S.F. Bamji: None. **B.N. Radde:** None. **P. Muluhngwi:** None. **C.M. Klinge:** None. **C. Corbitt:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.06/Q5

Topic: E.01. Neuroendocrine Processes

Title: Perinatal ethanol exposure alters the hypothalamus-pituitary-thyroid axis

Authors: *A. J. HOLDERMAN[1], R. LAWRENCE[2]; [1]Viterbo Univ., Amherst, WI; [2]Biol., Viterbo Univ., La Crosse, WI

Abstract: Developmental ethanol exposure is the leading cause of preventable developmental disabilities and produces dysfunction similar to hypothyroidism. Alcohol exposure has been linked to alterations in thyroid function, however, the specific location of the hypothyroid-pituitary-thyroid (HPT) axis and epigenetic mechanism has not been identified. We hypothesized the perinatal ethanol exposure would cause a change in the competency of the HPT axis. To determine this, we measured circulating TSH of the blood and TSH-β gene expression in the thyrotrophs of the anterior pituitary. A three trimester ethanol exposure model was used to best approximate human exposures. Pregnant Long Evans rats were divided into three groups: ethanol treated, intubated (stressed) control, and no treatment control. Once the pups matured, the blood, pituitary glands, and thyroid glands were collected. Using an Elisa assay kit, circulating levels of TSH were significantly elevated in the ethanol treatment group compared with the intubated control suggesting perinatal ethanol exposure mimics congenital hypothyroidism disorder potentially due to epigenetic alterations in TSHβ gene.

Disclosures: **A.J. Holderman:** None. **R. Lawrence:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

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Program#/Poster#: 73.07/Q6

Topic: E.01. Neuroendocrine Processes

Support: NIH grant RO1 NS57823

NIH grant P30 GM103398

Title: Neurokinin 3 receptor associates with transcription factor c-Fos and nuclear proteins in paraventricular neurons of the hypothalamus following osmotic challenge in rats

Authors: *A. THAKAR[1], T. R. MORDHORST[2], F. W. FLYNN[2];
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Abstract: Neurokinin 3 receptor (NK3R) is a G protein-coupled receptor that is widely expressed in brain and colocalizes with vasopressinergic neurons in the paraventricular (PVN) and supraoptic nuclei of the hypothalamus. We demonstrated that in response to hyperosmolarity, membrane-bound NK3R are activated, internalized to the cytoplasm, and translocated to the nucleoplasm of PVN neurons. Using co-immunoprecipitation (Co-IP) we showed that in nucleus, NK3R forms a complex with P300/CBP-associated factor (PCAF) and

acetyl-H3K9, and modifies chromatin structure and gene transcription. We next sought to determine if proteins with DNA binding motif, i.e. transcription factors, combined with NK3R in this complex. Male Charles River Laboratory rats were maintained on water or 2% NaCl, and chow (n=5/group) for 3 days. The rats were euthanized, the brain sectioned using a vibratome, and the PVN was isolated via micro punch (0.94 mm dia). PVN punches from rats in the same treatment group were combined, homogenized, and the nucleoplasm isolated. PCAF was conjugated to magnetic beads and used to pull down nuclear protein complexes. After elution, samples were probed for NK3R and then the following nuclear proteins: PCAF, c-Fos, GCN5, and acetyl-H3K9. Co-IP results showed that PCAF pulled down NK3R, c-Fos, β-actin, and acetyl-H3K9 in PVN samples from rats maintained on 2% NaCl, but not in control rat samples. The membrane was also probed for secretory carrier membrane protein 5 (SCAMP5) and, as expected, this protein was not present in the pulled down samples. We identify that the transcription factor c-Fos is pulled down by PCAF along with NK3R in nuclear samples from rats maintained on 2% NaCl. These data indicate that in response to an osmotic challenge (2% NaCl) NK3R are activated and translocated to the nucleus where NK3R forms a complex with PCAF to acetylate histones. Acetylation of histones relaxes chromatin structure and would allow for the recruitment of transcription factor c-Fos to specific promoter sites. Hyperosmolarity increases vasopressin and c-fos gene expression (Kawasaki et al., 2009). Interestingly, AP1 site is present on vasopressin promoter and c-Fos mediated increase in vasopressin gene promoter activity has been shown (Yoshida et al., 2008). Overall, our results suggests that within the complex, the role of NK3R may be to further target the binding of transcription factor c-Fos at AP1 site on VP promoter via selective histone acetylation by PCAF.

Disclosures: **A. Thakar:** None. **T.R. Mordhorst:** None. **F.W. Flynn:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

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Program#/Poster#: 73.08/Q7

Topic: E.01. Neuroendocrine Processes

Support: Max Planck Society

Title: Neuromorphological reconstruction using Brainbow reveals novel target regions innervated by magnocellular hypothalamic neurons in zebrafish larvae

Authors: *U. HERGET[1], A. GUTIERREZ-TRIANA[2], S. RYU[1];
[1]Max Planck Inst. For Med. Res., Heidelberg, Germany; [2]Ctr. for Organismal Studies, Heidelberg, Germany

Abstract: In vertebrates, a specialized group of hypothalamic neurons innervates the neurohypophysis, releasing arginine vasopressin (Avp) or oxytocin (Oxt) into the bloodstream. These neurons are called magnocellular due to their soma size, and are located mainly in the paraventricular (PVN) and supraoptic (SON) nuclei in rodents. Many studies in rodents have described important features of magnocellular neurons, yet their projections aside from pituitary innervation remained elusive. Recent advances have demonstrated the presence of central amygdala innervation by Oxt-producing cells in rats, but a comprehensive projectome analysis of magnocellular neurons is currently lacking since magnocellular neurons are difficult to access due to their deep location in the mammalian brain. Zebrafish larvae offer the possibility to investigate the entire brain in intact and living animals without the need for surgery or sectioning and therefore provide an opportunity to analyze structural and functional features of readily accessible hypothalamic neurons. In this work, we analyzed the 3D morphology of cells producing Avp or Oxt using genetic tools to specifically express Brainbow in the PVN-homologous region in larval zebrafish, the neurosecretory preoptic area (NPO). Brainbow was targeted to NPO cells using both an Oxt promoter and a conserved regulatory element of the transcription factor Orthopedia. Using inducible Cre recombination, the Brainbow cassette consisting of transgenes coding for different fluorescent proteins (XFPs) covering multiple colors is randomly recombined to express a different XFP in neighboring cells, thereby allowing the spectral separation of intermingled fibers. This approach shows the different long-range projections, which are difficult to analyze in rodent brains due to the need for histological sectioning. 3D reconstruction in Amira using a skeletonization plugin revealed the morphology of individual cells and their innervated target regions. Preliminary results show that Oxt-positive projections can reach into the tectum, the hypothalamus, and the telencephalon. Some broadly innervating Oxt-positive cells feature projections that cross the midline to the contralateral side. Our results suggest novel target regions innervated by these cells in several parts of the brain, providing insights into the potential structural basis of the many functions suggested for Oxt neurons. The detailed neuromorphological and hodological characterization of magnocellular cells established in this work provides the basis for the first comprehensive projectome analysis of Avp or Oxt-producing neurons in vertebrates.

Disclosures: U. Herget: None. **A. Gutierrez-Triana:** None. **S. Ryu:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: E.01. Neuroendocrine Processes

Support: CONACYT grants 127777,

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PAPIIT-UNAM 216214

Title: Hypertonicity-sensitive theta oscillations in hypothalamic paraventricular nucleus and intracerebral projecting axon-collaterals of individual vasopressinergic magnocellular neurons using *in vivo* extracellular recording and juxtacellular labeling and neuroanatomy methods

Authors: *V. S. HERNANDEZ, M. M. MARQUEZ, E. VAZQUEZ-JUAREZ, L. ZHANG; Dept. of Physiology, Fac. of Medicine, Natl. Autonomous Univ. of Mexico, Mexico City, Mexico

Abstract: Conventional neuroanatomical, immunohistochemical and *in vitro* recording and labeling methods may fail to detect long range intracerebral-projecting axons of vasopressinergic (AVP) magnocellular neurosecretory neurons (MNNs) of the hypothalamic paraventricular nucleus (PVN). Here, by using *in vivo* extracellular recording and juxtacellular labeling, we show that MNNs possessed multi-axon-like processes and axonal collaterals branching very near to the somata, projecting intracerebrally to areas including the medial preoptical area, suprachiasmatic nucleus, lateral habenula, medial and central amygdala and to the conducting systems such as stria medullaris, the fornix and the internal capsule. Axon-collaterals were detected to express vesicular glutamate transporter 2. Under basal condition, local field potential (LFP) recorded inside PVN showed spontaneous theta rhythms (4-6Hz). Hypertonicity induced increase in power and frequency. Furthermore, *in vivo* activation of AVP-MNNs by hypertonic saline administration produced significant reduction of freezing behavior and an increase in active escaping, measured as climbing, rearing and displacement, during live cat exposure. Modified Fos expression patterns during fear processing in hypothalamus, amygdala, thalamus and LHb were also observed. Our data demonstrate that AVP-MNNs possessed multiple axon-like processes and the long-range intracerebral projection is a more common feature of the MNNs, than an “occasional” phenomenon as previously thought. These magnocellular AVP-glutamatergic non-canonical pathways found here may constitute a part of the central motivational circuit activated under multifaceted stress coping. The origin, physiological significance of the theta rhythms recorded inside the PVN and its modification by hypertonicity remain to be determined.

Disclosures: **V.S. Hernandez:** None. **M.M. Marquez:** None. **E. Vazquez-Juarez:** None. **L. Zhang:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

Support: NIH grant RO1 NS57823

NIH grant P30 GM103398

Title: Perinuclear organization of neurokinin B terminals at NK3 receptor expressing neurons in the paraventricular nucleus

Authors: T. R. MORDHORST, R. GRENVIK, A. THAKAR, *F. W. FLYNN;
Neurosci Prgm, Univ. Wyoming, Laramie, WY

Abstract: The tachykinin neurokinin 3 receptor (NK3R) is expressed by the majority of vasopressin magnocellular neurons in the paraventricular nucleus of the hypothalamus (PVN). Injections of NK3R antagonists prevent the release of vasopressin and c-Fos expression in response to hypotension and hyperosmolarity. Neurokinin B (NKB) is the endogenous tachykinin with the highest affinity for NK3R while substance P (SP) has a much lower affinity. Previous reports indicate that NKB terminals and fibers are found in the PVN, but their detailed relationship to NK3R-expressing neurons, most likely vasopressinergic, was not described. We used double label immunohistochemistry to identify the innervation of NK3R by NKB and SP. Images of NK3R expressing neurons in the posterior magnocellular PVN were captured using confocal laser scanning microscopy. A region of interest (ROI) was drawn around the cluster of NK3R magnocellular neurons and the area of NKB fluorescence was measured in the ROI and an equal area in the perinuclear area that was devoid of NK3R soma. There was limited NKB immunoreactivity in the NK3R ROI. NKB immunofluorescence in the perinuclear area surrounding the ROI was approximately 4x greater than that measured in the ROI containing NK3R expressing neurons. NKB immunofluorescence was 10x greater in the dorsal ROI and dorsal perinuclear area than in the corresponding ventral regions. Within the NK3R expressing ROI, very few NKB terminals contacted NK3R soma or dendrites. In contrast, SP immunoreactive terminals distributed throughout the area of NK3R expressing neurons. This anatomical arrangement suggests that SP may activate NK3R. To test this hypothesis, CHO cells

were transfected with a NK3R plasmid and calcium imaging was used to test the effects of SP, NKB, and senktide, a NK3R agonist. NKB and senktide (1 nM) elicited a large calcium response, but SP had no effect. In addition, we tested the morphological effects of application of SP, NKB, and senktide on transfected and nontransfected CHO cells. NKB and senkitde (0.01, 0.1, 1.0 nM) caused a morphological rearrangement of transfected CHO but not in non transfected cells; SP had no effect. These results indicate that while SP, rather than NKB, terminals are proximal to NK3R, SP does not appear to activate NK3R. Oldfield and Silverman (Brain Res Bull, 1985, 14, 143-57) previously reported that limbic system structures project to the PVN in a perinuclear fashion and the dense NKB terminals surrounding NK3R expressing neurons may arise from these structures. Furthermore, the anatomical relationship of NKB and NK3R suggests a local volume transmission mode of NKB action at NK3R expressing vasopressin neurons in the PVN.

Disclosures: T.R. Mordhorst: None. **R. Grenvik:** None. **A. Thakar:** None. **F.W. Flynn:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.11/Q10

Topic: E.01. Neuroendocrine Processes

Support: DA013185

Title: A novel DiI staining methodology to examine effects of estradiol on dendritic spine morphology in the arcuate nucleus of the female rat

Authors: *L. M. RUDOLPH, P. E. MICEVYCH;
Dept. of Neurobio., Univ. of California Los Angeles, Los Angeles, CA

Abstract: In female rats, estradiol promotes morphological changes in the arcuate nucleus of the hypothalamus (ARH) critical for lordosis behavior. Specifically, dendritic spine formation in ARH neurons is required for estradiol-induced sexual receptivity. This estradiol induction of lordosis behavior is mediated by two changes in dendritic spine morphology. First, estradiol benzoate (EB) rapidly induces the formation of immature, filapodial spines within ~4 h, a process initiated by membrane signaling. Secondly, there is a shift from immature filapodial to mature mushroom spines 30-48 h after EB treatment. The increase in the proportion of mushroom spines coincides with the period of maximal lordosis behavior, suggesting that it is

not the initial formation of spines, but the maturation of dendritic spines that is responsible for the exhibition of sexual receptivity. While it is clear that mature spines in the ARH are necessary for lordosis, the pre-and post-synaptic roles in synapse formation, maturation, and stabilization are unclear. Furthermore, current methods used to examine dendritic morphology of arcuate neurons have multiple limitations: Golgi histology uses toxic chemicals and stains a random assortment of cells, crystal implantation of the lipophilic tracer 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) involves an extensive incubation period (e.g., months) for dye transport, and DiI labeling using gene guns (diolistics) is costly. To examine the morphology of dendritic spines in the ARH and circumvent these issues, we developed a novel technique of fluorescently labeling neurons using, DiI. DiI crystals were dissolved in ethanol and further diluted in phosphate-buffered saline (PBS). Perfused and fixed brains were blocked and sectioned at 200 μm on a vibratome and incubated in the DiI solution for 1 h, rinsed, and incubated overnight in PBS at 37°C. Sections were mounted and coverslipped with glycerol, sealed, and stored in the dark at 4°C until analysis with confocal microscopy. This technique allows for assessment of spine structures without additional equipment or prolonged incubation times, and can potentially be combined with immunocytochemistry to assess changes in spinogenesis and spine morphology in the ARH to understand the molecular mechanisms of estradiol-induced, spine-dependent expression of female sexual receptivity.

Disclosures: L.M. Rudolph: None. **P.E. Micevych:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.12/Q11

Topic: E.01. Neuroendocrine Processes

Support: Jeffress Foundation

Title: The effect of diet-induced obesity on estradiol-induced hormonal surges in female rats

Authors: *S. BLYTHE[1], A. E. GONZALEZ-IGLESIAS[2], R. BERTRAM[3], G. WHITWORTH[1], N. TOPORIKOVA[1];
[1]Biol., Washington & Lee Univ., Lexington, VA; [2]Neurosci., [3]Mathematics, Florida State Univ., Tallahassee, FL

Abstract: Over the past 20 years, obesity has become a major global health crisis, as it is associated with a number of medical problems including reduced fertility. Indeed, obesity in

women can lead to irregularities in the menstrual cycle, polycystic ovarian syndrome, and reduced conception rates. Therefore, we have characterized the effect of diet-induced obesity on the amplitude and timing of estradiol-induced hormonal surges. Juvenile female rats consumed a control diet or a high sugar, high fat (HSHF) diet from weaning. After 10 weeks on their respective diets, ovaries were removed from all of the animals (OVX) and then half were implanted with slow-release estradiol pellets (OVXE). At termination, HSHF rats weighed significant more than the control diets animals, and OVXE rats weighed more than OVX rats. HSHF animals also exhibited greater levels of abdominal fat and insulin resistance as compared to control rats. Finally, trunk blood samples were collected at 11 am, 2 pm, 5pm, 8 pm and 11 pm. Blood prolactin was measured with radioimmunoassay. In all OVXE rats the daily prolactin surge was induced; however, the amplitude of the surge was significantly lower in animals on HSHF diet. Aberrant prolactin signaling has been linked with reproductive difficulties and may represent a novel target for therapeutic intervention in obesity-related infertility.

Disclosures: **S. Blythe:** None. **A.E. Gonzalez-Iglesias:** None. **R. Bertram:** None. **G. Whitworth:** None. **N. Toporikova:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

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Topic: E.01. Neuroendocrine Processes

Support: NIH grant MH62677

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NIH grant AG29612

NIH grant OD11092

Title: Ovarian steroids regulate gene expression in the dorsal raphe of old female macaques

Authors: S. G. KOHAMA, *C. L. BETHEA, A. P. REDDY, H. F. URBANSKI;
Oregon Natl. Primate Res. Ctr., Beaverton, OR

Abstract: With extended lifespans in modern humans, menopause has become a significant risk factor for depression, anxiety, loss of cognitive functions, weight gain, metabolic disease, osteoporosis, cardiovascular disease and neurodegenerative disease. Clinical studies have found beneficial neural effects of ovarian steroid replacement (HRT) during the menopausal transition and data are emerging which suggest that these benefits can be sustained long-term. To further understand molecular underpinnings of the clinical studies, we used qRT-PCR to examine gene expression in the serotonergic dorsal raphe related to depression, cellular resilience and neurodegenerative disease. Old rhesus macaques (>21 yrs) maintained on a low fat and low sugar diet were ovariectomized (Ovx, surgically menopausal). Immediately after Ovx, groups of animals (n=5) were administered placebo, estradiol (E) or E + progesterone (E+P), which was continued for ~4 years. Significant beneficial changes were observed in 36 out of 48 genes examined. Increased expression was observed in mRNAs that encode proteins supporting serotonin neurotransmission (FEV.TPH2.CRFR2.UCN1), synapse assembly (NLGN3.NTRK.SNAP5.NCAM), glutamate neurotransmission (NMDA2a.AMPA2.GRM1), DNA repair (NBS1.NTHL.LIG4.RAD23.APEX1.DISS1), chaperones (HSP90B1.HSP60.HSP27), ubiquinases (UBE1.UBE2D3.UBE3A) and transport motors (KIF5.DCTN4.DYNCL1). Decreased expression of two mRNAs encoding proteins involved in neuropathology was also observed (APP.PSEN1). The data show that HRT maintains beneficial effects on gene expression in the serotonin system of old macaques after long-term treatment. Alternatively, the loss of ovarian steroids leads to decreased transcription at the gene level that may in turn lead to many of the observable neural deficits in serotonin target systems.

Disclosures: **S.G. Kohama:** None. **C.L. Bethea:** None. **A.P. Reddy:** None. **H.F. Urbanski:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

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Program#/Poster#: 73.14/Q13

Topic: E.01. Neuroendocrine Processes

Support: CONACYT CB 176393

SALUD-2012-1-179506

Title: The hormone prolactin is a novel survival factor for the retinal pigment epithelium through anti-oxidant actions

Authors: S. THEBAULT, *A. MARTINEZ-TORRES, R. MELÉNDEZ GARCÍA, D. ARREDONDO ZAMARRIPA, N. ADÁN, E. ARNOLD, G. BAEZA CRUZ, J. R. RIESGO-ESCOVAR, B. ORDAZ, F. PEÑA-ORTEGA, C. CLAPP;
INB-UNAM, Queretaro, Mexico

Abstract: Retinal pigment epithelial (RPE) cells form a polarized monolayer that is adjacent to the photoreceptors and indispensable for vision. Degeneration of RPE cells results in retinal disorders such as age-related macular degeneration. Because a major feature of this disease is the increased formation of reactive oxygen species (ROS), the identification of endogenous factors able to protect RPE cells from oxidative stress is important. Cell transplant strategies have potential therapeutic value for such disorders but an inadequate supply of donor cells limits their therapeutic success; therefore, identifying factors that help RPE cells to proliferate could provide a renewable source of cells for transplantation. Since we detected the hormone prolactin (PRL) and its receptor in RPE cells and previous studies reported PRL to be a survival factor, we postulated that RPE cells are a target for the PRL trophic effect. Using a cell line derived from human RPE (ARPE-19), we showed that conditioned medium from ARPE-19 monolayers contains PRL and that a specific anti-PRL antibody or a competitive PRL receptor antagonist reduces ARPE-19 cell viability. Also, we found that PRL prevented both the reduction in viability and proliferation and the increase in apoptosis of ARPE-19 cells induced by the oxidant hydrogen peroxide. ARPE-19 cells were also protected from hydrogen peroxide when PRL was applied after the oxidant. We further observed that PRL up-regulated the mRNA levels of the anti-oxidant glutathione S-transferase and reduced intracellular ROS levels in the presence of the oxidant. Because we detected the Transient Receptor Potential Melastatin 2 (TRPM2) channels in ARPE-19 cells and Ca2+ influx through TRPM2 in response to ROS promotes cell death, we examined the role of TRPM2 in the protective effect of PRL. TRPM2 inhibition by 3-aminobenzamide or TRPM2 silencing siRNA protected ARPE-19 cells from hydrogen peroxide-induced apoptosis. PRL also prevented the hydrogen peroxide-induced intracellular Ca2+ increase. Notably, RPE cells from mice lacking the PRL receptor showed reduced levels of glutathione S-transferase and higher levels of ROS and apoptosis than RPE cells from wild-type animals. This latter effect was exacerbated in aging animals. Taken together, these results indicate that PRL is a novel endogenous survival factor for the RPE that is able to protect it from oxidative damage.

Disclosures: S. Thebault: None. **A. Martinez-Torres:** None. **R. Meléndez García:** None. **D. Arredondo Zamarripa:** None. **N. Adán:** None. **E. Arnold:** None. **G. Baeza Cruz:** None. **J.R. Riesgo-Escovar:** None. **B. Ordaz:** None. **F. Peña-Ortega:** None. **C. Clapp:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.15/Q14

Topic: E.01. Neuroendocrine Processes

Support: SNF and ZHIP

Title: Amylin induces neurogenesis-like processes in the AP of adult rats

Authors: *C. LIBERINI[1], C. NEUNER BOYLE[2], T. A. LUTZ[3];
[1]Univ. of Zurich, Zürich, Switzerland; [2]vetsuisse faculty, Institue of Vet. Physiol., University of Zurich, Switzerland; [3]vetsuisse faculty, institue of Vet. Physiol., university of Zurich, Switzerland

Abstract: The area postrema (AP) is a sensory circumventricular organ characterized by extensive fenestrated vasculature and neurons which are capable of detecting circulating signals of osmotic, cardiovascular, immune and metabolic status. The peptide-hormone amylin is co-secreted with insulin by pancreatic β-cells in response to nutrient stimuli. Next to its role in the inhibition of glucagon secretion and in delaying gastric empting, amylin acts as a satiation signal by activating specific amylin-sensitive neurons in the AP. Moreover, amylin has been shown to promote the formation of neuronal projections originating from the AP. Thanks to the development of next-generation sequencing (NGS) technologies, systematic transcriptome analysis is now a highly sensitive approach to investigate changes in genetic expression. Here, RNA-sequencing was used to assess the influence of amylin on gene expression in the rat AP. Animals were treated with vehicle (0.1 mL/injection volume, i.p.), amylin (20 μg/kg, i.p.) and the amylin antagonist AC187 (500 μg/kg, i.p), and rat APs were collected under different feeding situations (e.g., fasting and re-fed). Our findings revealed that amylin affects several biological pathways, the most representative being neurogenesis, nervous system development, neuron generation and differentiation, synaptic transmission and cell-cell signaling. Several target genes identified by the NGS analysis were independently validated using RT-qPCR. NeuroD1 (Neuronal Differentiation 1) was strongly up-regulated (15 fold change) in the AP of adult rats after amylin treatment and this response was successfully blocked by the administration of AC187. Gabrb3 Gamma-Aminobutyric Acid (GABA) A Receptor, Beta 3), Gabra2 (GABA-A Receptor, Apha 2) and Gabrd GABA-A Receptor, delta) were also up-regulated in the same conditions; on the other hand, WNT4 (Wingless-Type MMTV Integration Site Family, Member) was consistently down-regulated by amylin treatment in food-deprived rats. Overall, because those genes were differentially expressed in the AP of adult rats, this suggests that amylin might mediate neurogenesis-like processes during adulthood in the AP.

Disclosures: C. Liberini: None. **C. Neuner Boyle:** None. **T.A. Lutz:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.16/Q15

Topic: E.01. Neuroendocrine Processes

Support: Grant-in-Aid for Scientific Research (B) (25293045)

Title: Characterization of the HAP1-immunoreactive cells in the subiculum and retrohippocampal formation in rat

Authors: *G. WROBLEWSKI, M. N. ISLAM, R. FUJINAGA, M. R. JAHAN, C. MATSUO, J. NEMOTO, K. TANAKA, K. ISHII, A. YANAI, K. SHINODA;
Yamaguchi Univ. Sch. of Med., Ube, Japan

Abstract: Huntingtin-associated protein 1 (HAP1) is a neural huntingtin interactor with putative protective functions against some neurodegenerative diseases. HAP1-immunoreactive (ir) neurons are abundant in the hypothalamus and some limbic regions and have also been characterized in the hippocampus as “sporadically lurking HAP1-ir (SLH)” cells, which express estrogen receptor (ER) α and are likely involved in memory function. In the present study, using paraformaldehyde-fixed frozen sections of adult male Wistar rat brains (9W), HAP1 expression was analyzed immunohistochemically in two additional memory-associated regions: (1) the subiculum and (2) the retrohippocampal formation, including the subiculum-associated complex (SAC) (post-, pre- and parasubiculum), and medial/lateral entorhinal, perirhinal and retrosplenial cortices. HAP1-ir cells were present in all regions and confirmed as neurons through double immunostaining with NeuN. Most intriguing, however, were two distinct HAP1-ir cell-dense zones. The first was a thin, continuous line of intensely-stained neurons in layer V of SAC just inferior to the lamina dissecans, most containing HAP1-ir stigmoid bodies (STB) in the cytoplasm. While no such layer was present in the bordering subiculum, entorhinal and retrosplenial cortices, the cells spanned the entire SAC and were thus dubbed the “SAC HAP1-ir layer (SACHL).” The second was a band of densely-packed, light-to-moderately-stained neurons without clear STBs, which coincided with layers II/III of the retrosplenial granular cortex (RSG) and was termed the “HAP1-ir retrosplenial granular band (HRGB).” Both structures were immunonegative for calbindin (CB), parvalbumin (PV), Wfs1 and ERα, with only minor exception. Solitary, intensely HAP1-ir neurons were also scattered through nearly every layer of the subiculum and retrohippocampal and neighboring cortices, but in general, none of the cells immunostained for CB, PV, or Wfs1. Most of the sporadic ERα-ir neurons, however, coexpressed HAP1 - particularly in superficial layers - resembling SLH cells in the hippocampus. The current study clearly showed that HAP1 is a distinct marker for both the SAC

and RSG, where the SACHL and HRGB, respectively, were first identified. The former could be the site of grid, border and head direction cells linked to proper orientation and cognition, while the latter might be associated with the precuneus in human, recently recognized as a compromised area in Alzheimer's disease. Thus, due to putative HAP1 protection, the HAP1-ir neurons detected in these memory-associated brain regions should be more stable against apoptosis in neurodegenerative disease.

Disclosures: G. Wroblewski: None. **M.N. Islam:** None. **R. Fujinaga:** None. **M.R. Jahan:** None. **C. Matsuo:** None. **J. Nemoto:** None. **K. Tanaka:** None. **K. Ishii:** None. **A. Yanai:** None. **K. Shinoda:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.17/Q16

Topic: E.01. Neuroendocrine Processes

Title: Interaction between serum and brain insulin like growth factor-1 participates in recovery after brain injury

Authors: *A. SANTI[1,2], L. GENIS[1,2], A. TRUEBA-SAINZ[1,2], R. HERRO[1,2], T. NISHIJIMA[1,2,3], J. PIRIZ[1,2,4], I. TORRES-ALEMAN[1,2];
[1]Inst. Cajal, Madrid, Spain; [2]CIBERNED, Madrid, Spain; [3]Tokyo Metropolitan Inst., Tokyo, Japan; [4]Buenos Aires Univ., Buenos Aires, Argentina

Abstract: Brain insulin-like growth factor I (IGF-I) input arises from local sources (paracrine) and also from the periphery (endocrine), as this growth factor is taken up from the blood-stream. Because the functional significance of this dual origin of IGF-I neuroprotection is poorly understood, we examined the role of paracrine and endocrine IGF-I in the response to brain injury. We found that brain damage abrogated an inhibitory action that serum IGF-I exerted on the expression of brain IGF-I. Indeed, brain injury elicited increased IGF-I synthesis in the affected area. Furthermore, systemic administration of IGF-I to increase low serum IGF-I levels seen only in severely injured mice, lead to restored brain function. Collectively, these data indicate that brain injury reorganizes regulatory interactions between brain and circulating IGF-I to increase its trophic input in the affected region. These observations also support the use of serum IGF-I levels as a biomarker of brain injury severity.

Disclosures: **A. Santi:** None. **L. Genis:** None. **A. Trueba-Sainz:** None. **R. Herro:** None. **T. Nishijima:** None. **J. Piriz:** None. **I. Torres-Aleman:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.18/Q17

Topic: E.01. Neuroendocrine Processes

Support: Texas Garbey Foundation

UNTHSC seed grant

Title: Membrane associated androgen receptor associated with G-proteins

Authors: ***J. G. CONTRERAS**[1], B. SNYDER[2], S. HOLMES[2], R. L. CUNNINGHAM[2]; [1]UNTHSC, Fort Worth, TX; [2]UNTHSC, Ft. Worth, TX

Abstract: Men have a two-fold risk for Parkinson's disease (PD) than women. PD is typified by the loss of dopamine neurons in the substantia nigra. Our previous studies show that testosterone, the major male sex hormone, can increase oxidative stress, calcium influx, and cell death in dopamine neurons. Furthermore, these studies showed that the membrane impermeable testosterone (T-BSA) also caused oxidative stress and cell death in dopamine neurons. Therefore, we hypothesize that a membrane bound receptor coupled to a G-protein receptor binds testosterone and mediates testosterone's negative effects on dopamine neurons. To test our hypothesis we used co-immunoprecipitation of membrane fractions from a dopaminergic cell line, N27 cells, and substantia nigral tissue from male rats to determine if the androgen receptor is associated with g-proteins., such as Gαq or Gαo subunits. Our results show that the androgen receptor is associated with both Gαq or Gαo subunits in the membrane fraction of N27 dopaminergic cells and in substantia nigral tissue. Thus, our data indicates that testosterone may induce a cell signaling cascade originating from a Gαq subunit, leading to increased oxidative stress, calcium influx, and cell death in dopamine neurons

Disclosures: **J.G. Contreras:** None. **B. Snyder:** None. **S. Holmes:** None. **R.L. Cunningham:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.19/Q18

Topic: E.01. Neuroendocrine Processes

Support: FAPESP Grant 2014/18165-9

FAPESP Grant 2013/10706-8

CNPq Grant 476643/2012-0

Title: Maternal high fat diet consumption during pregnancy and lactation affects cholinergic anti-inflammatory pathway in offspring mice

Authors: T. BALIANI, S. F. LEMES, T. FANTE, A. REGINATO, C. M. SILVA, M. MILANSKI, A. S. TORSONI, *M. A. TORSONI;
Faculdade De Ciências Aplicadas/Unicamp, Limeira, Brazil

Abstract: Introduction: The acetylcholine binds to α7nAChR and reduce cytokines production through cholinergic anti-inflammatory pathway (CAP). The human obesity has been associated to increased inflammatory cytokines level and reduced nicotinic acetylcholine receptor (α7nAChR) expression in white adipose tissue (WAT). Maternal obesity predisposes offspring to obesity in the adulthood. We aimed to evaluate cholinergic anti-inflammatory pathway in mice of obese dams. Methods: Liver and WAT fragments were extracted from male offspring mice (Swiss) (28 days old-d28) of dams fed either control diet (SC-O) or high fat diet (HFD-O) during pregnancy and lactation. Leptin and cytokines levels, expression and phosphorylation of α7nAChR, JAK2/STAT3, and M1/M2 polarization of peritoneal macrophages (PM) were evaluated using ELISA, western blot, immunofluoresce and qRT-PCR, western blot, and immunofluorescence. Results: HFD-O mice presented higher body weight, WAT mass, blood leptin and TNFα level, and p-JNK1 in liver and WAT than SC-O mice. In addition HFD-O showed reduced expression of M2 markers (Chill3, IL-10, and ARG-1) compared to SC-O mice. α7nAChR expression, p-JAK2 and p-STAT3 were reduced in liver and WAT of HFD-O compared to SC-O mice. In SC-O mice α7nAChR protein was observed in both, macrophages (F4/80) and cells that did not express F4/80 protein, likely adipocytes and hepatocytes. However, the α7nAChR expression was not detected adipocytes of HFD-O mice. HFD-O mice showed reduced activation of CAP after challenger with either LPS (IP) or nicotine (ICV) compared to SC-O. Conclusion: HFD-O mice presented early inflammatory pathway activation and obesity, and reduced sensitivity of cholinergic anti-inflammatory pathway compared to SC-O mice.

Impair in cholinergic anti-inflammatory pathway can predispose to exacerbated inflammation and metabolic disorders in response to invaders and TLR4 activation

Disclosures: **T. Baliani:** None. **S.F. Lemes:** None. **T. Fante:** None. **A. Reginato:** None. **C.M. Silva:** None. **M. Milanski:** None. **A.S. Torsoni:** None. **M.A. Torsoni:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.20/Q19

Topic: E.01. Neuroendocrine Processes

Support: Royal Society of New Zealand Marsden Grant

Title: *In vivo* electrophysiological evidence demonstrating the anti-opioid effect of the neuropeptide FF system

Authors: *J. KIM, C. H. BROWN, G. M. ANDERSON;
Univ. of Otago, Dunedin, New Zealand

Abstract: Agonists of the neuropeptide FF receptors (NPFFR1 and NPFFR2) have been generically termed “anti-opioids” for their putative ability to block opioid function. However *in vivo* evidence for this has been limited to nociceptive tests, which are confounded by the pronociceptive effects of the NPFFR ligands. To elucidate the functions of the NPFFRs, we first identified and characterised a true, potent, and selective antagonist, called GJ14. Next, we used the vasopressin neurons of the supraoptic nucleus as a model to examine the anti-opioid function of the NPFFR ligand, RFamide related peptide-3 (RFRP-3). In extracellular single-unit recordings from urethane-anaethetised rats, the spontaneous firing rate of vasopressin neurons was significantly reduced by morphine (i.v. 30 ug/kg). This inhibition was virtually abolished by pretreatment with RFRP-3 (i.c.v. 12 nmol) and morphine sensitivity was recovered 10 min after RFRP-3 treatment. Control rats receiving 3 consecutive morphine treatments alone did not show any change in morphine sensitivity. RFRP-3 alone had no effect on vasopressin neuron firing rate. A challenging notion is that chronic opioid treatment triggers the upregulation of these anti-opioid systems, which in turn attenuates the effect of morphine, thereby producing tolerance. To test this hypothesis, rats were given a continuous dose of morphine (10 mg/kg/day) via osmotic mini pumps for 6 days. Vasopressin neuron reponses to morphine (i.v. 30 ug/kg) were virtually absent in morphine-infused rats, confirming morphine tolerance. Pretreatment with GJ14 (i.c.v. 50 nmol) increased the sensitivity to morphine in vasopressin neurons of tolerant rats. In

summary, this is the first evidence demonstrating an anti-opioid function *in vivo* using electrophysiology. Furthermore using our novel antagonist, we report convincing evidence that the NPFFRs are an important part of a genuine anti-opioid system that regulates opioid sensitivity.

Disclosures: J. Kim: None. **C.H. Brown:** None. **G.M. Anderson:** None.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.21/Q20

Topic: E.01. Neuroendocrine Processes

Support: 5R01MH050604

Title: Genetic variations in the glucocorticoid and mineralcorticoid receptor genes are associated with disrupted hypothalamic functional connectivity and elevated nocturnal cortisol secretion in depression

Authors: *K. D. SUDHEIMER, J. KELLER, R. O'HARA, G. MURPHY, A. F. SCHATZBERG;
Psychiatry, Stanford Univ., Palo Alto, CA

Abstract: Our group has recently demonstrated that patients with typical depression (NPMD) and patients with psychotic major depression (PMD) have reduced resting state hypothalamic functional connectivity (FC) with the subgenual cortex. Looser cortisol regulation, driven by genetic predispositions built into the structure of the cortisol receptors themselves, may play a key role in driving these reductions in FC. Here we demonstrate that a significant amount of the variance in hypothalamic connectivity to the subgenual cortex can be explained using measures of genetic variance in the two main cortisol receptors and measures of cortisol secretion during the evening hours Down-regulation of cortisol receptors is thought to play a key role in causing HPA axis dysregulation and producing the emotional/neurophysiological changes that accompany major depression. Genetic variability in cortisol receptors could increase/decrease susceptibility to these changes by altering the structure/sensitivity of the receptors. We have presented evidence that resting-state functional connectivity (FC) between the hypothalamus and the subgenual cortex (SGC) is disrupted in patients that have major depression with psychotic features and that these disruptions are associated with symptom severity. We hypothesized that genetic variability across the glucocorticoid receptor (GR) and mineralcorticoid receptor (MR)

would be associated decreases in hypothalamic FC to the SGC and elevated cortisol during the circadian nadir. 74 patients in 3 groups (healthy, major depression, psychotic major depression) had genetics data for 9 GR single nucleotide polymorphisms (SNP), and 14 MR SNPs. Backwards step-wise linear regressions were conducted to determine associations between genetic variance in GR or MR and hypothalamic FC to the SGC. The final GR regression model explained 36.2% of the variance in hypothalamic FC to the SGC $F(6,65)=7.72, p<0.001$. Higher cortisol, age, and 2 GR SNPs (rs41423247, rs2918419) were associated with more disrupted connectivity. A single GR SNP (rs12655166) was associated with less disrupted connectivity. The MR regression model explained 21.2% of the variance in connectivity $F(3,69)=7.46, p<0.001$. In this model, age and the MR SNP (rs5522) were associated with disrupted connectivity. These results indicate that genetic variability in the GR and MR genes could be affecting the neurophysiological networks associated with the symptoms and severity of depression.

Disclosures: **K.D. Sudheimer:** None. **J. Keller:** None. **R. O'Hara:** None. **G. Murphy:** None. **A.F. Schatzberg:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Corcept Theraputics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Corcept Theraputics.

Poster

073. Neuroendocrine Anatomy and Physiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 73.22/R1

Topic: E.01. Neuroendocrine Processes

Support: Conacyt grant 220598

DGAPA grant IG 200314

Title: Glucocorticoid receptors in the arcuate nucleus mediate a fast feedback of corticosterone secretion

Authors: *L. A. LEON, D. HERRERA MORO CHAO, M. C. BASUALDO SIGALES, R. M. BUIJS;
Univ. Nacional Autonoma De Mexico, Distrito Federal, Mexico

Abstract: Precise control of the level of glucocorticoids in the circulation is necessary to regulate several physiological processes as the metabolism of glucose and lipids and the response to stress. Although the endocrine portion of the Paraventricular nucleus of the hypothalamus (PVN) has been proposed as one of the major centers involved in glucocorticoid regulation, there is ample evidence of a key role of the autonomic portion of the PVN in the control of the release of corticosterone (Cort) from the adrenal gland. We demonstrate here that the autonomic neurons of the PVN lack Cort receptors (GR) therefore we hypothesized the PVN may require an additional system to sense hormonal changes in the bloodstream. The arcuate nucleus of the hypothalamus (ARC), is a circumventricular organ that possesses GR type I and II and projects monosynaptically to the PVN. We investigated whether the ARC is able to detect and produce fast changes of Cort in the circulation in order to elucidate a neural pathway involved in the control of the adrenal production of Cort. We retrodialyzed pharmacological agonists or antagonists for GR type I and II bilaterally into the ARC and took blood samples to measure Cort and ACTH. We found that the GR antagonist produced a fast and sustained increase in Cort levels which was not accompanied by changes in total ACTH in the morning. On the other hand, when dialyzed in the afternoon, when the circadian peak is produced, we found a clear effect of the GR agonist on the levels of Cort. In addition, we retrodialyzed GR antagonist unilaterally to characterize the activation of the PVN after the infusion and examined the expression of per1 and CYP11A1 in the adrenal. The result was a clear unilateral activation of the PVN and an increased expression of the analyzed genes in one adrenal gland. Taken together, our findings demonstrate the importance of the arcuate nucleus to maintain accurate Cort levels, and suggest an autonomic neural pathway responsible for controlling the production of GC in the adrenal gland. Grant support: Conacyt 220598 DGAPA IG 200314

Disclosures: **L.A. Leon:** None. **D. Herrera Moro Chao:** None. **M.C. Basualdo Sigales:** None. **R.M. Buijs:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.01/R2

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

Support: NIH Grant U01 AA018279

Loyola Research Funding Award

Title: PARP-1 regulates neuroinflammatory phospholipase A2 (PLA2) and HMBG1 in alcohol-binged rat adult brain slice cultures

Authors: *M. A. COLLINS[1], N. TAJUDDIN[1], E. J. NEAFSEY[1], H.-Y. KIM[2];
[1]Mol. Pharmacol., Loyola Univ. Chicago, Maywood, IL; [2]Lab. of Mol. Signaling, NIAAA, Bethesda, MD

Abstract: Chronic/subchronic alcohol (ethanol) binges cause brain neurodegeneration via neuroinflammatory/neuroimmune mechanisms that likely require protein activators (cytokines; Crews et al. 2013) and lipid mediators (PLA2, arachidonic acid (ARA) and possibly downstream eicosanoids; Collins et al. 2014). Focusing on the lipid mediator processes, we have found elevated levels of Ca+2-dependent PLA2 (cPLA2 IVA), phosphorylated (activated) p-cPLA2, secretory PLA2 (sPLA2 IIA), aquaporin-4 (AQP4), and poly [ADP-ribose] polymerase-1 (PARP-1) that correlate with neurodegeneration in binge alcohol-treated rat organotypic hippocampal-entorhinal cortical slice cultures of adult brain age (~60 d; Tajuddin et al. 2014). Excessive ARA release by PLA2 could cause neurotoxic oxidative stress. Also, nuclear PARP-1 has DNA repair functions, but its overactivation can trigger a regulated necrotic neuronal death route (parthanatos). In that respect, we have reported that a potent PARP-1 inhibitor (PJ-34) affords neuroprotection in the alcohol-binged slice cultures. Here we examined whether PARP-1 might modulate levels of the above PLA2's and AQP4 (which is known to be linked to neuroinflammatory edema). The results with binge alcohol indicate that PARP-1 does not fuel elevations in AQP4; however, the nuclear enzyme is evidently upstream of cPLA2 and sPLA2, since PJ-34 completely suppresses both to about control levels. This may be the first evidence in brain for PLA2 levels being coupled to enhanced PARP-1. Furthermore, relevant to cytokine involvement, the PARP-1 inhibition significantly antagonized alcohol-induced increases in high mobility box group 1 (HMBG1) protein, an endogenous toll-like receptor-4 agonist associated with secretion of proinflammatory IL-1β. Thus, interconnected pro-oxidative necrotic cascades of neuroinflammatory phospholipid-derived and protein mediators induced by neurotoxic binge alcohol exposure could be dependent, at least in part, on a "master" regulator, PARP-1. Crews et al. (2013) Biol. Psychiat. 73:602-612; Collins et al. (2014) Mol. Neurobiol. 50:239-245; Tajuddin et al. (2014) PLoS ONE 9(7):e101223.

Disclosures: **M.A. Collins:** None. **N. Tajuddin:** None. **E.J. Neafsey:** None. **H. Kim:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.02/R3

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

Support: NIH P50 MH103222

Lundbeck Research USA

Title: Prenatal LPS exposure preferentially increases kynurenine pathway metabolism in the fetal brain

Authors: *F. M. NOTARANGELO, K. S. WONS, R. SCHWARCZ;
Maryland Psychiatric Res. Center, Dept of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Maternal infection during pregnancy increases the risk for the offspring to develop a broad spectrum of psychiatric disorders, including schizophrenia (Brown and Patterson, 2011). Prenatal exposure of mice to lipopolysaccharide (LPS) leads to brain and behavioral abnormalities in the offspring, but the underlying mechanisms are still unknown. The kynurenine pathway (KP) of tryptophan degradation is strongly regulated by the immune system (Saito et al., 1992) and may constitute a molecular link between immune activation and psychiatric diseases. Notably, the KP contains several neuroactive metabolites, including kynurenic acid (KYNA), an antagonist of the α7 nicotinic acetylcholine receptor and the N-methyl-D-aspartate (NMDA) receptor, and, in a competing branch of the pathway, the free radical generator 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN), an agonist of the NMDA receptor. In the present study, we evaluated the effect of an intraperitoneal injection of a low dose of LPS (100 μg/kg) on gestational day 15 on KP metabolism in CD1 mice. To this end, kynurenine, KYNA and 3-HK levels were determined in maternal plasma and brain, as well as in placenta and fetal brain, 4 and 24 h after the immune challenge. No differences in maternal body weight or number of embryos were observed between controls and LPS-treated mice. Moreover, KP metabolite levels were slightly elevated in placenta 4 and 24 h after LPS injection, but the increases did not reach statistical significance. In contrast, kynurenine levels were significantly increased in the fetal brain 4 h after exposure to LPS ($p<0.01$, $n=3$), compared to the control group, and were still elevated after 24 h ($p<0.05$, $n=4$). A similar trend was observed for KYNA and 3-HK levels, with significant increases seen 4 h after the LPS injection ($p<0.01$, $n=3$). Interestingly, this increase in KP metabolism was not observed in the maternal brain at this low dose of LPS, indicating a higher susceptibility of the developing brain to infection. These results suggest that even relatively modest prenatal immune activation, by increasing KP metabolism specifically in the fetal brain, may have detrimental long-term consequences during postnatal development. In particular, the observed elevation in KYNA levels in the fetal brain may increase the risk of developing psychiatric disorders later in life (Pocivavsek et al., 2014).

Disclosures: **F.M. Notarangelo:** None. **K.S. Wons:** None. **R. Schwarcz:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

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Program#/Poster#: 74.03/R4

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

Support: SAF2009-08136 from Ministerio de Ciencia e Innovación of Spain

USP-BS-APP03/2014 from Universidad CEU San Pablo and Banco de Santander

Title: Pleiotrophin differentially modulates microglial response and astrocytosis in LPS- and amphetamine-induced neuroinflammation

Authors: *G. HERRADON[1], C. PEREZ-GARCÍA[1], E. GRAMAGE[1], R. FERNÁNDEZ-CALLE[1], M. FERRER-ALCÓN[2], M. URIBARRI[2], M. VICENTE-RODRÍGUEZ[1]; [1]Pharmacol. Lab, CEU San Pablo Univ., MADRID, Spain; [2]BRAINco Biopharma, Derio (Bilbao), Spain

Abstract: Pleiotrophin (PTN) is a cytokine that is upregulated in CNS pathologies characterized by chronic neuroinflammation and has been shown to exert a pivotal role in some of these disorders including Parkinson's disease and drug addiction. Amphetamine-induced striatal astrocytosis is increased in PTN genetically deficient (PTN-/-) mice. In contrast, PTN is known to induce inflammatory mediators and its expression levels are significantly reduced by administration of anti-inflammatory drugs. Therefore, it is important to clarify the modulatory role of PTN in neuroinflammation. For this purpose, we have now comparatively studied the astrocytic and microglial responses to lipopolysaccharide (LPS) and amphetamine treatments in PTN-/- mice and transgenic mice overexpressing PTN in the brain cortex (PTN-Tg). In immunohistochemistry studies, we found that a single, very low, dose of LPS (0.5 mg/kg) induced a significant increase in the number of glial fibrillary acidic protein (GFAP)-positive cells only in the prefrontal cortex (PFC) of wild type (WT) mice compared to saline-treated animals. Amphetamine treatment (10 mg/kg, 4 times, every 2 h) did not alter the astrocytic response in the PFC of WT and PTN-Tg mice but it was found significantly down-regulated in PTN-/- mice. The data suggest that LPS- and amphetamine-induced astrocytosis is not directly regulated by endogenous PTN although alterations in the levels of endogenous PTN may cause compensatory mechanisms with some impact on the astrocytic response. Interestingly, we found that 0.5 mg/kg LPS is not sufficient to induce microgliosis in the PFC of PTN-/- and WT mice. However, LPS induced a significant increase in the number of CD11b-positive cells in PTN-Tg mice. On the other hand, amphetamine caused a significant up-regulation of microgliosis only in

the PFC of PTN-Tg mice although this effect was significantly lower compared with LPS-treated PTN-Tg mice. The data demonstrate that the endogenous levels of PTN are critically important to trigger microglial response after different insults and suggest that the known upregulation of PTN levels in different pathologies of the CNS may underlie the characteristic neuroinflammation of these disorders.

Disclosures: G. Herradon: None. **C. Perez-García:** None. **E. Gramage:** None. **R. Fernández-Calle:** None. **M. Ferrer-Alcón:** None. **M. Uribarri:** None. **M. Vicente-Rodríguez:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.04/R5

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

Title: Physical deformations of perivascular and meningeal spaces and immune cell activation caused by cortical spreading depression in the mouse

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Abstract: About 16% of the population suffers from migraine, and about a third of those have migraines that are preceded by abnormal sensory perception called aura. This aura is likely due to a brain phenomenon described in rodents as cortical spreading depression (CSD), where cascading neighboring neurons in the cortex become transiently hyperactive and then inhibited. When CSD is induced in rodents, it propagates over the cortex in minutes (at about 2-3 min/mm), and causes activation of meningeal nociceptors only after a delay (20-45 min), that is similar to the delayed onset of headache post aura in humans (20-60 min). It is not known how CSD activates meningeal nociceptors. In this study, we attempt to begin answering this question. Using time lapse multiphoton microscopy in live transgenic mice, we determine the effect of CSD on resident immune cells in the meninges, perivascular space, and intracranial tissues, and the effect of CSD on the these tissues. Using a thin-skull window, we observed CSD-induced phenotypic changes in macrophages, dendritic cells, and T-cells in each cranial layer (the pia, subarachnoid, dura, and skull endeosteum) as well as deformation of perivascular spaces. These findings provide a sequence of anatomical and cellular events that can lead to activation of meningeal nociecptors by CSD.

Disclosures: **A. Schain:** None. **R. Burstein:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

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

Support: NIH Grant NIH/NINDS R01NS080844

Newborn Medicine Funds from the Department of Pediatrics, University of Mississippi Medical Center

Title: Disturbance of neurobehavioral performance and dopaminergic neuronal injury in the adult rat brain following neonatal exposure to interleukin-1beta

Authors: ***L.-W. FAN**[1], L.-T. TIEN[4], Y. PANG[1], S. LU[1], H. ZHU[1], J. SHEN[1], J. P. SHAFFERY[2], X. DAI[3], A. J. BHATT[1], R. D. SAVICH[1];
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Abstract: Our previous studies showed that interleukin-1β (IL-1β), an inflammatory cytokine, plays important roles in dopaminergic neuronal injury in the neonatal rat brain. To examine whether neonatal IL-1β exposure has long-lasting effects in adult rats, brain injury (postnatal day 70, P70) and motor functions (from P7 to P70) were examined after an intracerebral injection of IL-1β (1 μg/kg) in P5 Sprague-Dawley male rat pups. Our results showed that neonatal IL-1β exposure resulted in disturbances of motor behaviors including hyperactivity in the open field task, and dysfunction in the vibrissa-elicited forelimb-placing, movement initiation, pole and tapered/ledged beam walking tests. However, the impaired motor functions were spontaneously recovered on P70. On the other hand, neonatal IL-1β exposure caused a sustained activation of microglia and elevated IL-1β, IL-6 and Interferon-γ levels, as well as reduced tyrosine hydroxylase expression in the substantia nigra of P70 rats. These results indicate that exposure to IL-1β in the neonatal rats may cause sustained brain inflammatory responses, as well as long-lasting damage to the dopaminergic system in the adult rat brain. The finding that IL-1β induced brain injury was very similar to that induced by lipopolysaccharide (LPS) as we previously reported suggests that IL-1β may play a critical role in mediating brain injury associated with perinatal infection/inflammation.

Disclosures: **L. Fan:** None. **L. Tien:** None. **Y. Pang:** None. **S. Lu:** None. **H. Zhu:** None. **J. Shen:** None. **J.P. Shaffery:** None. **X. Dai:** None. **A.J. Bhatt:** None. **R.D. Savich:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

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

Support: NIH R01NS031758

NIH R01NS082283

NIH P20 GM103620

NIH P20 GM103548

Title: Diet-induced obesity prolongs neuroinflammation in latent herpes simplex virus-(HSV)-1 infected mice by increasing microglia activation and infiltrating monocytes

Authors: K. A. M. WHITE[1], S. R. HUTTON[2], *J. M. WEIMER[1], P. A. SHERIDAN[3]; [1]Children's Hlth. Res. Ctr., Sanford Res., Sioux Falls, SD; [2]Univ. of North Carolina Neurosci. Ctr., [3]Gillings Sch. of Global Publ. Hlth., Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Obesity results in increased inflammation, susceptibility to infection, and has been linked with anxiety and cognitive impairment. Children raised in low socioeconomic status (SES) have diets higher in fat, are at greater risk for anxiety and learning difficulties, and are seropositive for herpes simplex virus-(HSV)-1 at a younger age than their higher SES counterparts. Previous studies from our lab have sought to determine how obesity alters neuroinflammation in mice latently infected with HSV-1. These studies found obesity prolongs inflammation in brains of latent HSV-infected mice, resulting in increased anxiety. In this study, we sought to determine the contribution of infiltrating monocytes to prolonged neuroinflammation in obese, latently infected mice. Weanling C57Bl/6 and $CCR2^{RFP/+}/CX3CR1^{GFP/+}$ mice were placed on a 10% low fat (LF) diet one week prior to receiving an intranasal HSV-1 or mock infection. Fourteen days post infection, mice were randomized to remain on the LF diet or switch to a 45% high fat (HF) diet. At various points following infection, the number and phenotype of infiltrating cells was determined using immunohistochemistry and flow cytometry. In comparison to both latently infected mice on the LF diet and uninfected mice on LF and HF diets, latently infected mice on the HF diet had

greater increased microglial activation and infiltration of inflammatory $CCR2^+$ monocytes in the hypothalamus and dentate gyrus. There was increased VCAM staining indicating an increase in the adhesion molecule expression in the areas of monocyte infiltration ($p<0.05$). Infiltrating monocytes also produced proinflammatory cytokines demonstrating that, along with activated microglia, monocytes contribute to sustained neuroinflammation in latently infected obese mice and may play a significant role in the anxiety phenotype of these mice. Together, these mice provide a useful, testable model to study the biobehavioral effects of obesity and latent HSV-1 infection in regards to anxiety and may provide a tool for studying diet intervention programs in the future.

Disclosures: K.A.M. White: None. **S.R. Hutton:** None. **J.M. Weimer:** None. **P.A. Sheridan:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

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Program#/Poster#: 74.07/R8

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

Support: Action On Hearing Loss

MRC

Rosetree Trust

Title: Acute Hyperbilirubinaemia induces Endoplasmic Reticulum (ER) Stress and NFκB driven neurodegeneration in a mouse model

Authors: *E. SCHIAVON[1], J. L. SMALLEY[2], I. D. FORSYTHE[2];
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Abstract: Jaundice is caused by high levels of unconjugated bilirubin in the blood, that when severe cause toxicity and damage in susceptible brain areas (including the cerebellum and auditory brainstem). This results in ataxia and hearing loss and is also associated with cognitive deficits in humans with kernicterus. We developed an acute, rapid onset and reversible model of bilirubin toxicity using CBA/Ca mice. The model exhibits similar neurological problems to those seen in human BIND syndrome (Bilirubin-Induced Neurological Dysfunction) and correlated with deficits in auditory brainstem response (ABR) which is an *in vivo* measure of hearing

function. We compared whole genome gene expression measurements in the human neuronal SHSY5Y cell line and mouse brain tissue following bilirubin exposure, and combined this with Connectivity Mapping to gain mechanistic insights into the toxicity process. We found that bilirubin induces Endoplasmic Reticulum (ER) stress and up-regulation of strategic genes involved in the Unfolded Protein Response (UPR). Additionally there was striking similarity between bilirubin-induced changes and the well-known ER-stress-inducing chemical, Thapsigargin. This mouse model provides a reliable and rapid induction of hyperbilirubinaemia. The results show that bilirubin toxicity causes an inflammation in the auditory brainstem driven by non-canonical activation of NF-kB (Nuclear factor-kB). These observations highlight the role of neuroinflammation in mechanisms of central auditory disease and suggest convergence of common pathological signalling with neurodegeneration.

Disclosures: E. Schiavon: None. **J.L. Smalley:** None. **I.D. Forsythe:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

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Program#/Poster#: 74.08/R9

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

Support: Defense Threat Reduction Agency (DTRA)

Title: IL-6 signaling exacerbates brain damage progression but reduces peripheral sickness measures following soman-induced status epilepticus

Authors: *J. IRWIN[1], L. SHUMWAY[1], J. CHANDLER[1], K. LAITIPAYA[1], T. FERRARA-BOWENS[1], M. WEGNER[2], E. A. JOHNSON[1];
[2]Comparative Pathology, [1]USAMRICD, Gunpowder, MD

Abstract: Severe neuropathology and behavioral impairment are a result of prolonged status epilepticus (SE) caused by organophosphorus compounds such as soman (GD). GD, a potent acetylcholinesterase inhibitor, causes prominent cell death in the hippocampus, thalamus, amygdala and piriform cortex, leading to the activation of the neuroinflammatory cascade. Neuroinflammation can exacerbate tissue injury or promote healing, depending on the intricate interaction of multiple cells, inflammatory factors and receptors as the injury progresses, a fact that has complicated the development of effective neuroprotective therapies for CNS injury models. IL-6 is a signaling protein known to be involved in many inflammatory and immune functions in both the periphery and brain and can produce both pro- and anti-inflammatory

effects. To determine which role IL-6 may play in central and peripheral GD effects, this study used wild type and background-matched IL-6 knockout mouse strains to identify changes acute neurodegeneration, mortality, convulsion onset and weight loss following GD exposure. The results showed that attenuation of IL-6 signaling does reduce brain injury following GD exposure though peripheral sickness measures were exacerbated as indicated by a significantly greater 24 hour weight loss compared to wild type controls. These data suggest that the pro- and anti-inflammatory effects of IL-6 may be highly dependent on central/peripheral localization of signals. Regardless, IL-6 inhibition does appear to be a viable neuroprotective strategy though further studies will have to better characterize the varying effects of IL-6 in different organ systems.

Disclosures: J. Irwin: None. **L. Shumway:** None. **J. Chandler:** None. **K. Laitipaya:** None. **T. Ferrara- Bowens:** None. **M. Wegner:** None. **E.A. Johnson:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

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

Support: NICHD Grant R01HD069562

Title: Cerebellar inflammation and dysfunction in a rabbit model of cerebral palsy

Authors: *S. NARAYAN, Z. ZHANG, E. NANCE, S. KANNAN;
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Abstract: Increasing evidence shows that apart from its motor function, the cerebellum is involved in higher cognitive functions and has functional connectivity to the frontal cortex. We have previously shown that intrauterine lipopolysaccharide (LPS) exposure induced white matter injury in the cerebrum, along with increased microglia and astrocytes activation, resulting in cognitive, motor, and behavior deficits that are consistent with clinical findings of cerebral palsy (CP). We have also shown that targeting activated microglia and astrocytes using dendrimer nanoparticles to deliver anti-inflammatory agents to these cells on PND1 resulted in improvement of their motor function. However, little is known about the presence and role of neuroinflammation in the cerebellum following a maternal intrauterine insult. Given recent data implicating cerebellar involvement in other delayed behavioral and developmental diagnoses, such as autism and cerebral palsy, the goal of this study is to determine whether exposure to

maternal inflammation *in utero* results in inflammation in the immature cerebellum and whether this is associated with motor and cognitive dysfunction in the juvenile rabbit. Time-pregnant New Zealand white rabbits underwent a laparotomy at gestation day 28. Endotoxin group received 8000 EU of LPS injection along the length of the uterus and control saline group received saline injection. Cerebellum from control saline and endotoxin groups were harvested at postnatal day (PND) 1 and 5. Presence and activation of microglia were detected by Iba1 immunohistochemistry staining, morphology characterized using Neurolucida, and inflammatory cytokine profiles were quantified by PCR and ELISA. Cerebellar learning was determined using the eye-blink conditioning response and compared between the CP and age matched control groups. We found that there was increased microglial 'activation,' as evidenced by increased numbers and change in morphology of microglia in the cerebellar white matter and the cerebellar nuclei (deep grey matter) in the PND 1 endotoxin kits when compared with the control saline kits. The potential for targeting activated microglia and inflammation in the cerebellum will be evaluated using dendrimer nanoparticles and localization, and distribution of these nanodevices in the cerebellum will also be determined.

Disclosures: **S. Narayan:** None. **Z. Zhang:** None. **E. Nance:** None. **S. Kannan:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

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

Support: CNPq

ANR-10-IAIHU-06

FAPESP

Title: Cannabidiol reduces LPS-induced activation and oxidative stress in primary microglial culture via PPARgamma receptor

Authors: *A. B. SONEGO[1], J. E. SEPULVEDA-DIAZ[4], P. P. MICHEL[4], E. A. DEL-BEL[2], F. S. GUIMARAES[3], R. RAISMAN-VOZARI[4];
[2]Morphology, Physiol. and Basic Pathology, [3]Pharmacol., [1]Univ. of Sao Paulo, Ribeirao Preto, Brazil; [4]Inst. du Cerveau et de la Moelle Epinière, Sorbonne Univ. UPMC UM75 INSERM U1127, Paris, France

Abstract: Cannabidiol (CBD), the major non-psychoactive constituent from Cannabis sativa, has antioxidant and anti-inflammatory properties which could be of potential therapeutical interest. Peroxisome proliferator-activated receptor (PPAR)-γ is a putative effector protein for CBD actions. Here, we aimed to further explore the mechanisms underlying CBD anti-inflammatory effects using a system model of mouse microglial cells, i.e., the resident immune cells of the brain. More specifically, we wished to determine (i) whether CBD was able to diminish oxidative stress in microglial cells stimulated by the bacterial inflammogen LPS (10 ng/ml) and (ii) whether PPARγ was involved in this effect. Our data show that CBD was able to diminish substantially the activation of microglial cells exposed to LPS (10 ng/ml) for 24 h. More specifically, CBD treatment led to reduced cellular expression of the microglial cell marker Iba-1 and to decreased production of reactive oxygen species measured with the fluorogenic probe CELLROX. Likewise, CBD limited the translocation of the p65 subunit of the major proinflammatory nuclear transcription factor NFκB after an acute stimulation of 30 min with LPS. Interestingly, all anti-inflammatory effects of CBD were antagonized by the PPARγ antagonist GW9662. Altogether, our data indicate that CBD attenuates LPS-induced oxidative stress in microglial cells through the activation of PPARγ and the repression of NFκB translocation.

Disclosures: A.B. Sonego: None. **J.E. Sepulveda-Diaz:** None. **P.P. Michel:** None. **E.A. Del-Bel:** None. **F.S. Guimaraes:** None. **R. Raisman-Vozari:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.11/R12

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

Support: NIH

AHA

Title: Toxic role of prostaglandin E2 receptor EP1 after intracerebral hemorrhage in mice

Authors: *J. WANG[1], X. ZHAO[1], T. WU[1], C. CHANG[1], H. WU[1], X. HAN[1], Q. LI[1], Y. GAO[1], T. MARUYAMA[3], J. ZHANG[2];
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Abstract: Inflammatory mechanisms mediated by prostaglandins may contribute to the progression of intracerebral hemorrhage (ICH)-induced brain injury, but they are not fully understood. In this study, we examined the effect of prostaglandin E2 receptor EP1 (EP1R) activation and inhibition on brain injury in mouse models of ICH and investigated the underlying mechanism of action. ICH was induced by injecting collagenase into the striatum of middle-aged male and female mice and aged male mice. Effects of selective EP1R agonist ONO-DI-004, antagonist SC51089, and nonspecific Src family kinase inhibitor PP2 were evaluated by a combination of histologic, magnetic resonance imaging (MRI), immunofluorescence, molecular, cellular, and behavioral assessments. EP1R was expressed primarily in neurons and axons but not in astrocytes or microglia after ICH induced by collagenase. In middle-aged male mice subjected to collagenase-induced ICH, EP1R inhibition mitigated brain injury, brain edema, cell death, neuronal degeneration, neuroinflammation, and neurobehavioral deficits, whereas its activation exacerbated these outcomes. EP1R inhibition also was protective in middle-aged female mice and aged male mice after collagenase-induced ICH. EP1R inhibition also reduced oxidative stress, white matter injury, and brain atrophy and improved functional outcomes. Histologic results were confirmed by MRI. Src kinase phosphorylation and matrix metalloproteinase-9 activity were increased by EP1R activation and decreased by EP1R inhibition. EP1R regulated matrix metalloproteinase-9 activity through Src kinase signaling, which mediated EP1R toxicity after collagenase-induced ICH. We conclude that prostaglandin E2 EP1R activation plays a toxic role after ICH through mechanisms that involve the Src kinases and the matrix metalloproteinase-9 signaling pathway. EP1R inhibition could be a novel therapeutic strategy to improve outcomes after ICH.

Disclosures: **J. Wang:** None. **X. Zhao:** None. **T. Wu:** None. **C. Chang:** None. **H. Wu:** None. **X. Han:** None. **Q. Li:** None. **Y. Gao:** None. **T. Maruyama:** None. **J. Zhang:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

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

Title: Quantitative SPECT/CT imaging of neuroinflammation in neurodegenerative disease models

Authors: T. HUHTALA, A.-M. ZAINANA, M. BJÖRKMAN, J. RYTKÖNEN, T. PARKKARI, *O. M. KONTKANEN, P. J. SWEENEY, A. NURMI;
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Abstract: Neuroinflammation is associated with neurodegenerative diseases, including multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), and traumatic brain injury (TBI). Activation of the mitochondrial Translocator Protein (TSPO) in neuronal tissue is linked with neuroinflammation and TSPO ligands can be applied to image the progression of neuroniflammation *in vivo* using SPECT or PET. For *in vivo* SPECT/CT imaging of neuroinflammation, 123I-CLINDE was selected as a radioligand. In previous preclinical and clinical studies 123I-CLINDE has been demonstrated to be specific TSPO ligand of inflammation. In the current studies, we assessed inflammatory changes in preclinical models of neuropathic pain, MS, and HD. To study neuropathic pain, the neuritis model was used. Freund's Complete Adjuvant was applied in Surgicel band around sciatic nerve in rats. Tactile and thermal allodynia were evaluated on Days 3, 7 and 9 after the induction. For MS, EAE induction was performed with the delivery with MOG1-125 in female Dark Agouti rats. To study neuroinflammation in HD, the R6/2 mouse strain, was selected. All animal models were selected because they have a well-characterized phenotype that reflects the disease state. In the neuritis model, there was a significant increase in ligand binding in injured hind leg on Day 4 and Day 5 after induction. In the EAE model, animals were assessed on Day 14 and Day 28 post inoculation. A clear accumulation of 123I-CLINDE was seen in cerebellum, brainstem and spinal cord in contrast to naïve animals where no ligand binding in the CNS was observed. Inflammation in the R6/2 mouse model of HD was studied at the age of 10 weeks. A Significant increase in 123I-CLINDE binding was seen in cortex, hippocampus, striatum, medulla and pons indicating activation of inflammation on those areas. In this study, we present usability of 123I-CLINDE combined with SPECT/CT imaging and gamma counter analysis to monitor progression on inflammation in several preclinical rodent models. These data provide support for the use of 123I-CLINDE as a non-invasive, readily translatable method to monitor inflammation.

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Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

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

Support: NIH Grant NS34179

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Title: CD36 signaling in resident brain cells mediates post-ischemic brain injury by promoting free radical production in infiltrating neutrophils

Authors: *L. GARCIA-BONILLA, G. RACCHUMI, M. MURPHY, J. ANRATHER, C. IADECOLA;
Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: The class B scavenger receptor CD36 has been implicated in a wide variety of CNS pathologies (Exp Neurol, 261:633, 2014). After cerebral ischemia, CD36 signaling triggers an inflammatory response associated with brain leukocyte infiltration and damage (J Neurosci, 28:1649, 2008). However, it is unknown if CD36 in resident brain cells or peripheral leukocytes is responsible for its damaging effects. To address this question, lethally irradiated wild type (WT) or CD36−/− mice were transplanted with CD36−/− or WT bone marrow (BM) to generate chimeric mice with selective CD36 expression either in BM-derived cells or in brain cells. Five weeks later, mice underwent transient middle cerebral artery occlusion (MCAO; n=12/group) and injury volume was assessed 3d later in cresyl violet-stained brain sections. CD36−/− mice transplanted with WT BM (WT⇒CD36−/−) had reduced infarct volumes (18±1 mm3; Mean±SE), not different from those of CD36−/−⇒CD36−/− mice (14±1 mm3, p>0.05). Conversely, CD36−/−⇒WT mice had infarcts similar to those of WT⇒WT mice (48±8 mm3 vs. 50±9 mm3, p>0.05). Thus, ischemic damage is independent of CD36 in peripheral leukocytes. Brain flow cytometry was used to determine whether the reduced infarct volume was associated with reduced leukocyte infiltration 3d after MCAO. As anticipated, in CD36−/−⇒CD36−/− mice, in which infarcts are reduced (-72%), post-ischemic leukocyte infiltration (CD45high cells) was attenuated (9±2 x103 vs. 31±7 x103 vs. cell/hemisphere; p0.05), in which infarcts are not reduced. In WT⇒CD36−/− mice, the neutrophil number (CD45high/CD11b+/Ly6G+ cells) in the infiltrate was larger than in CD36−/−⇒CD36−/− mice (2±0.3 vs. 7±1 x103; p0.05). Since neutrophils contribute to ischemic injury by producing reactive oxygen species (ROS) (J. Immunol, 193:253, 2014), we next asked if CD36 in brain cells is required for neutrophil ROS production after MCAO. Despite similar infiltration levels, the number of neutrophils producing ROS in WT⇒CD36−/− was reduced compared to CD36−/−⇒WT mice (15±4% vs. 28±4% dihydroethidium positive neutrophils; p< 0.05). We conclude that CD36 in resident brain cells is required for ROS production by neutrophils and for their neurotoxic effect in the post-ischemic brain. Efforts to suppress post-ischemic CD36 signaling in brain cells may provide new approaches to reduce brain injury by mitigating the harmful effects of oxidative stress.

Disclosures: L. Garcia-Bonilla: None. **G. Racchumi:** None. **M. Murphy:** None. **J. Anrather:** None. **C. Iadecola:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

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

Support: MOST103-2320-B-030-005-MY3 from the Ministry of Science and Technology of Taiwan

NIH/NINDS R01NS080844

Title: Neonatal lipopolysaccharide-induced long-lasting learning impairment and hippocampal injury was attenuated by IL-1 receptor antagonist in adult rats

Authors: *L.-T. TIEN[1], Y.-J. LEE[1], J.-A. LEE[2], L.-W. FAN[3];
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Abstract: We have previously reported that neonatal lipopolysaccharide (LPS) exposure resulted in inflammatory responses, as indicated by an increase in interleukin-1β (IL-1β) content, a hippocampal injury, and cognitive deficits in juvenile and female adult rats. The present study aimed to determine whether an anti-inflammatory cytokine, interleukin-1 receptor antagonist (IL-1ra), protects against the neonatal LPS exposure-induced inflammatory responses, hippocampal injury, and long-lasting learning deficits in adult rats. LPS (1 mg/kg) or LPS plus IL-1ra (0.1 mg/kg) was injected intracerebrally to Sprague-Dawley male rat pups at postnatal day 5 (P5). Neurobehavioral tests were carried out on P21, P49, and P70, while neuropathological studies were conducted on P71. Our results showed that neonatal LPS exposure resulted in learning deficits in rats, as demonstrated by a significantly impaired performance in the passive avoidance task (P21, P49, and P70), and reduced the hippocampal volume and number of NeuN+ cells (neurons) in the CA1 region of the middle dorsal hippocampus of P71 rat brain. LPS induced a sustained inflammatory response in the P71 rat hippocampus, as evidenced by increase in number of activated microglia and elevation levels of IL-1β contents. Neonatal administration of IL-1ra significantly attenuated LPS-induced long-lasting learning deficits, hippocampal injury, and sustained inflammatory responses in P71 rats. Our study demonstrates that neonatal LPS exposure causes a persistent injury to the hippocampus and results in long-lasting learning disabilities related to chronic inflammation in rats, and these effects can be attenuated with an IL-1 receptor antagonist.

Disclosures: **L. Tien:** None. **Y. Lee:** None. **J. Lee:** None. **L. Fan:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

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Program#/Poster#: 74.15/R16

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

Title: Sepsis stimulates gliogenesis but has no significant impact on neurogenesis in the dentate gyrus

Authors: *A. KUNZE[1], P. BLÜMEL[1], B. GRÜNEWALD[1,2], S. KEINER[1], F. WOITKE[1], O. WITTE[1], C. GEIS[1,2], C. REDECKER[1];
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Abstract: Sepsis is a major cause of mortality and morbidity in intensive care units. Acute and long-term brain dysfunctions have been frequently demonstrated in septic patients. Thereby, pathophysiology of sepsis associated encephalopathy (SAE) remains insufficiently elucidated although there is evidence for inflammatory processes involving endothelial activation, alterations of blood-brain barrier and neurotransmission. Recent experimental studies suggest that cellular dysfunction in neurogenic niches such as the subventricular zone may also contribute to SAE. In the present study, we address the question whether adult neurogenesis in the hippocampus is impaired in an animal model of sepsis. For sepsis induction, adult transgenic pNestin/GFP mice received intraperitoneal injection of faeces on day 0. The precursor cells were labelled either with the thymidine analog BrdU or retrovirus expressing red fluorescent protein on days -4 to -1 prior to sepsis induction. Using confocal microscopy we analyzed the number and location of new born neurons in the dentate gyrus at days 14 and 42 post-sepsis-induction (psi). On days 37 to 41, we investigated spatial memory of mice in a modified Morris water maze which is considered to be a hippocampal-dependent behavioral task. We found that i) the number of BrdU cells significantly increased 6 weeks psi, ii) sepsis mainly stimulates gliogenesis in the dentate gyrus whilst the number of new born neurons increases but without statistical significance, iii) mice reveal no significant impairment of spatial memory psi. iv) preliminary data point towards a disturbance of synaptic integration of new neurons psi. Taken together our study supports the perspective that sepsis has an impact on gliogenesis and neurogenesis in the hippocampus.

Disclosures: **A. Kunze:** None. **P. Blümel:** None. **B. Grünewald:** None. **S. Keiner:** None. **F. Woitke:** None. **O. Witte:** None. **C. Geis:** None. **C. Redecker:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.16/R17

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

Support: NRF-2014H1A2A1021693

Title: Role of thyroid transcription factor-1 in transcriptional regulation of heme oxygenase-1 under neuroinflammation condition

Authors: *B. JEONG[1], J. KIM[2], B. LEE[1];
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Abstract: Hypothalamic inflammation has emerged as a major driver of energy homeostasis dysfunction in both obesity and anorexia. Thyroid transcription factor (TTF-1) is a member of homeodomain-containing transcription factor family, and plays a role in transcriptional regulation of genes expressed in the thyroid, lung and brain. In this study, we found that TTF-1 plays an important role in the transcriptional regulation of heme oxygenase-1 (HO-1) in hypothalamic inflammation. HO-1 is an inducible isoform of the first and rate-limiting enzyme in heme degradation and is a well-known cytoprotective enzyme against inflammation. TTF-1 deficiency resulted in exacerbated inflammatory response in the rat astroglial C6 cells under the presence of tumor necrosis factor-alpha (TNF-α). We found expression of TTF-1 and HO-1 in the mouse hypothalamus by using immunohistochemistry. Furthermore, luciferase assays and chromatin immunoprecipitation assays showed that TTF-1 directly activated HO-1 transcription by binding to its binding domains on the HO-1 promoter. Consistent with these results, mRNA and protein level of HO-1 were increased by overexpression of TTF-1 but decreased by shRNA-mediated inhibition of TTF-1 expression in the C6 cells. Taken together, these results suggest that TTF-1 participates in the hypothalamic inflammation by regulating HO-1 transcription.

Disclosures: B. Jeong: None. **J. Kim:** None. **B. Lee:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.17/R18

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

Support: NIH DUE-0969568

NIH DUE-0850132

Lupus Foundation of Minnesota

Title: Relationship of serum complement component 3 and behavioral alterations differs between male and female lupus-prone MRL/MpJ-Faslpr/J mice

Authors: *M. J. LARSON[1], E. LUCKHARDT[2], A. FRANZ[3], L. SHIUE[5], C. QUINLAN[6], K. D. STRAND[4];
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Abstract: Systemic Lupus Erythematosus (SLE) is a multifaceted autoimmune, inflammatory disease of unknown etiology that targets joints, skin, heart, kidneys, lungs, blood vessels, and brain. Prevalence is difficult to determine, but is 6-10 times higher in women than men. As many as 80% of adults with SLE experience neuropsychiatric SLE (NPSLE), which comprises 19 syndromes including headache, cerebrovascular disease, seizure, psychosis, mood disorders, and changes in cognitive functioning. People may experience NPSLE in the absence of systemic disease flairs. MRL/MpJ-Fas^{lpr}/J mice (MRL-*lpr*) resulted from a spontaneous autosomal retrotransposon insertional mutation that produces a non-functional variant of the pro-apoptotic cell surface receptor *Fas* and are a model of SLE and NPSLE. They develop autoantibodies to Smith antigen, nuclear proteins, and native single- and double-stranded DNA, but also exhibit changes in cognitive function, emotionality, and motivated behavior associated with autoimmunity. GWAS have suggested variants in some complement proteins may be associated with SLE. The complement cascade comprises several proteins that aid in destroying bacteria and stimulating immune response cells. Inactive complement proteins in the blood are activated by antigen-antibody complexes which initiates a series of cleavage and recruitment steps of other proteins in the cascade. C3 is a central molecule in the complement system that is involved in all three of the complement pathways. It can cause tissue damage by releasing histamine from mast cells to contract smooth muscle and increase vascular permeability. To evaluate C3 as a potential biomarker of NPSLE syndromes, we measured learning, memory, anxiety, anhedonia, and exploration, along with serum C3 using ELISA, in male and female MRL-*lpr* and control strains at pre-symptomatic through severely symptomatic stages of SLE. In males, a decrease in serum C3 was associated with reduced exploratory drive, impaired avoidance learning, and increased preference for sucrose solution. In females, decreased serum C3 levels were associated with impaired memory by novel object recognition and increased preference for sucrose solution. In

group comparisons, C3 levels were reduced in both male and female MRL-*lpr*s compared to MRL-+/+ congenic controls, but were only lower in MRL-*lpr*s compared to MRL-*lpr*2 animals in the males. C3 may be a marker for some NPSLE syndromes, but appears to differ between males and females. This research should be expanded to evaluate serum complement proteins and NPSLE syndromes in humans.

Disclosures: **M.J. Larson:** None. **E. Luckhardt:** None. **A. Franz:** None. **L. Shiue:** None. **C. Quinlan:** None. **K.D. Strand:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.18/R19

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

Support: Research Manitoba

the Children's Hospital Research Institute of Manitoba

Title: Gestational diabetes mellitus in pregnant rats induces chronic neuroinflammation, synaptic degradation and behavioral changes in the offspring

Authors: B. VUONG[1,2], G. ODERO[1,2], M. STEVENSON[1,2], S. M. KERELIUK[2,3], T. J. PEREIRA[2,3], V. W. DOLINSKY[2,3], *T. M. KAUPPINEN[1,2,3];
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Abstract: Gestational diabetes mellitus (GDM) is the most common complication of pregnancy and population health studies have linked it to impaired cognitive performance in the offspring. GDM and diets containing excess fats and sugars promote inflammatory responses. Prolonged inflammation can impair the neuronal circuitry development in the fetus resulting in lifelong effects on cognitive functions. We hypothesized that GDM causes adverse inflammatory responses in the fetus. This inflammatory environment could disturb the fine-tuning of developing neuronal networks impairing the neurocognitive abilities of the offspring. We induced GDM by feeding female rats a “junk food” diet high in sucrose and fatty acids 6 weeks prior to mating and throughout their pregnancy. Fetal (18.5E) and 15 week-old (young adult) offspring from GDM and lean dams were examined. The neurocognitive abilities of 15 week-old offspring were evaluated in Open field, Morris Water Maze and with Novel Object Recognition

test, and the brains from both age groups were analyzed by immunohistochemistry. Complementary *in vitro* experiments involved analyzing microglial responses to elevated levels of glucose and/or fatty acids. Offspring from GDM dams showed atypical explorative behaviour in open field test. The reduced neurocognitive performance directly correlated to maternal glucose imbalance (fasting blood glucose) during gestation. Analysis of brain tissues derived from the fetal and 15 week-old offspring of GDM dams showed increased astroglial GFAP expression, increased microglial morphological activation, and reduced expression of synaptic vesicle protein. Consumption of a post-weaning high fat and sucrose diet by the GDM offspring further promoted GDM-induced abnormalities. Cultured microglia exposed to high glucose and/or fatty acids transformed into activated, amoeboid morphology, significantly increased nitric oxide production, and changed cytokine release profile. Both *in vitro* and *in vivo* data demonstrate that GDM induces chronic inflammatory responses in the brain of the offspring that persist into young adulthood. Microglia culture experiments confirmed that excess glucose and/or fatty acids induce pro-inflammatory responses. Detrimental pro-inflammatory responses combined with impaired microglial neurotropic functions could explain synaptic degradation that contributes to behavior changes, and memory and learning impairments in the offspring.

Disclosures: B. Vuong: None. **G. Odero:** None. **M. Stevenson:** None. **S.M. Kereliuk:** None. **T.J. Pereira:** None. **V.W. Dolinsky:** None. **T.M. Kauppinen:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.19/R20

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

Title: Antidepressant effect of Agmatine against lipopolysaccharide induced Depression in mice

Authors: *N. B. GAWALI, V. BULANI, A. CHOWDHURY, P. KOTHAVADE, M. GURSAHANI, A. JUVEKAR;
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Abstract: Preclinical and clinical studies suggest that activation of immuno-inflammatory and oxido- nitrosative stress pathways play major role in the pathophysiology of depression. Increasing evidence has indicated that immune challenge by bacterial lipopolysaccharide (LPS) induces depression and neuro-inflammatory response. The present study was undertaken to investigate the effect of Agmatine pre-treatment on lipopolysaccharide-induced depression,

neuro-inflammation and oxido-nitrosative stress in mice. Mice were challenged with the endotoxin lipopolysaccharide (0.83 mg/kg, i.p.). Agmatine (20 and 40 mg/kg, i.p.) was administered 30 min before LPS. LPS-treated animals presented an increase in immobility time in the forced swimming test (FST) and tail suspension test (TST) when compared to control group 24 hours after endotoxin administration. It was observed that Imipramine (IMI-10 mg/kg, i.p.) and Agmatine at both doses prevented and reversed LPS-induced alterations in the FST and TST. IL-1β content was increased 24 hours after LPS administration in hippocampus (HC) and prefrontal cortex (PFC). IMI and Agmatine prevented and reversed LPS-induced increase in IL-1β level in HC and PFC. IMI and Agmatine pre-treatment prevented LPS evoked oxidative/ nitrosative stress via improving reduced glutathione level along with reduction in the lipid peroxidation and nitrite level in HC and PFC. These results indicate that Agmatine pre-treatment provided protection against LPS-induced depressive which may be mediated by repression of pro-inflammatory cytokines as well as oxido-nitrosative stress in HC and PFC. This study suggests that Agmatine may be an important therapeutic target in the treatment of depression and other pathophysiological conditions which are closely associated with neuro-inflammation and oxido-nitrosative stress.

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Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.20/S1

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

Support: by Novartis funding, study # CFTY7220DUSNC10T

Title: Modulation of S1P receptors at the Blood Brain Barrier can influence its response to inflammatory stimuli

Authors: *S. F. SPAMPINATO[1,2], B. OBERMEIER[2], A. COTLEUR[2], A. LOVE[2], Y. TAKESHITA[2,3], R. RANSOHOFF[2];
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Abstract: The importance of the blood brain barrier (BBB) to maintain the principal functions of the brain is well known. Recently the involvement of the barrier in several Central Nervous System(CNS) affections has been demonstrated. In multiple sclerosis (MS) a leaky BBB, together with demyelinated multifocal lesions, constitutes a typical hallmark of the pathology. Several therapeutic approaches have been developed to reduce the migration of leukocytes towards the BBB aiming to reduce the demyelination area. Recently a sphingosine-1 phosphate (S1P) analog, fingolimod, has been introduced in MS treatment. The immunomodulator, acting as functional antagonist for the S1P1 receptor, prevents the egress of T cells from the lymph node, and reduces the onset of new lesions. S1P receptors are as well expressed on endothelial cells and astrocytes, that together with pericytes, are the main cellular constituents of the BBB. Here we investigated whether key BBB properties could be affected by S1P receptor modulation, addressing in particular the role exerted by the immunomodulator fingolimod. For this purpose, we used an *in vitro* co-cultured model to investigate the effects of S1P receptors modulation on endothelial cells and astrocytes. We either examined endothelial cells and astrocytes independently, or in a more physiological condition in which physical contact between the two cell types was enabled. S1P receptors modulation rescued endothelial cells from death upon cytokine challenge, either directly, or indirectly, acting on astrocytes, through the release of trophic factors. When exposed to TNFα and IFNγ in fact astrocytes released granulocytes and macrophages colony growing factor (GM-CSF), able to prevent endothelial cells death induced by cytokines exposure. Finally in an *in vitro* BBB model incorporating shear stress, S1P receptor modulation reduced leukocytes migration across the endothelial barrier. The data here reported point out the possibility that fingolimod, already in use for the treatment of relapsing remitting forms of MS, could also act at the BBB reinforcing it and reducing the damage induced by inflammatory condition and the access of leukocytes in the CNS.

Disclosures: S.F. Spampinato: None. **B. Obermeier:** None. **A. Cotleur:** None. **A. Love:** None. **Y. Takeshita:** None. **R. Ransohoff:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.21/S2

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

Title: Characterization of lipopolysaccharide-induced TLR4 neuroinflammatory signaling and the effects of fentanyl in CHME-5 human microglial cells

Authors: *L. K. FIGUEROA[1], S. DAS[2], C. W. STEVENS[1], R. L. DAVIS[1];
[1]Pharmacol. & Physiol., [2]Anat. and Cell Biol., Oklahoma State Univ. Ctr. For Hlth. Sci., Tulsa, OK

Abstract: Neuroinflammation is a key mediator of neuronal damage observed in various neuropathologies, thus, understanding pro-inflammatory pathways of human glia is important in identifying potential pharmacological strategies to modulate inflammatory signaling in these cells. Microglia-derived cytokines and chemokines are instrumental in neuroinflammation and several pro-inflammatory mediators activate these cells to include bacterial lipopolysaccharide (LPS) that signals through toll-like receptor 4 (TLR4). Even though peripheral TLR4 signaling is well defined, human microglial TLR4 signaling is not. Furthermore, recent studies suggest the ability of opioid agents to modulate immune function in peripheral and central nervous system cells. Therefore, our goals are to characterize the molecular mechanisms of LPS-induced TLR4 signaling, and, elucidate the effects of the mu opioid receptor (MOR) agonist, fentanyl, on this signaling in human microglial cells, CHME-5. In keeping with these goals, LPS-induced tumor necrosis factor (TNF) gene expression, both message, and, intracellular and secreted protein levels were upregulated in a time-dependent manner. Additionally, analysis of phosphorylated NF-κB p65 revealed optimal activation with 100 ng/ml LPS O55:B5 for 90 min in CHME-5 cells. Lastly, dose-dependent co-exposure of LPS with fentanyl significantly upregulated LPS-induced NF-κB binding activity at 10 μM. To date, our data suggest that CHME-5 human microglial cells serve as an experimental model for analyzing the inflammatory actions of human microglial TLR4 signaling, which may be exacerbated by opioid compounds, specifically, fentanyl. Thus, we have also initiated studies to elucidate the effects of LPS, fentanyl and other pharmacological agents on key TLR4 signal transduction proteins. Together, these ongoing studies are expected to further elucidate the mechanisms of LPS-induced TLR4 signaling and the effects of opioid agents in human microglia.

Disclosures: L.K. Figueroa: None. **S. Das:** None. **C.W. Stevens:** None. **R.L. Davis:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.22/S3

Topic: C.03. Parkinson's Disease

Support: FAPESP

CAPES

CNPQ

Title: Neuronflammation markers in the substantia nigra associated to L-DOPA-induced dyskinesia

Authors: *E. DEL BEL[1], M. .-. -. -. BORTOLANZA[2], R. KWIATKOSKI[3], F. E. PADOVAN-NETO[3];
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Abstract: The major pathological feature of Parkinson's disease (PD) is the progressive degeneration of the nigrostriatal system. It leads to the loss of neurons in the substantia nigra (SN) and subsequent loss of striatal dopaminergic - terminals. The degeneration of the nigrostriatal pathway contributes to the clinical motor symptoms: tremor, rigidity, bradykinesia and postural instability. Long-term l-3,4-dihydroxyphenylalanine (L-DOPA) treatment in PD patients leads to development of abnormal involuntary movements or L-DOPA-induced dyskinesia. As the main target of the nigral dopamine (DA) neurons, the striatum has received much attention in regard to understanding the pathophysiology of LID. It suggests that several dopaminoceptive structures in the brain that are likely to be affected by the exogenously produced DA have received little attention. These regions might play a key role in mediating those L-DOPA-induced abnormal behaviors. Recently, we described an increase of inflammatory markers (GFAP, OX42, iNOS and COX2) in the striatum of hemiparkinsonian rats presenting L-DOPA-induced dyskinesia. To go further on identify neuroinflammatory response specifically related to L-DOPA-induced dyskinesia we report the changes in GFAP, OX42, iNOS and COX2 expression in the SN reticulata/compacta of four groups of rats: hemiparkinsonian, hemiparkinsonian chronically treated with 7-Nitroindazole (7NI- a neuronal nitric nNOS inhibitor), hemiparkinsonian chronically treated with L-DOPA exhibiting dyskinesia, and hemiparkinsonian chronically treated with L-DOPA preceded by 7NI and without exhibiting dyskinesia. Non-dyskinetic parkinsonian animals displayed a rather normalized expression of GFAP and OX42, and no expression of iNOS and COX2 in the studied brain regions. Dyskinetic animals are distinguished by significant changes of the OX42 and GFAP in the SN-compacta ventral and lateral. Also, there was a striking increased expression of the OX42 in the SN-reticulata. The overexpression of GFAP and OX42 in these brain regions correlates with L-DOPA-induced dyskinesia severity. NOS inhibition prevented L-DOPA-induced dyskinesia and either GFAP or OX42 increased expression. Glial cells accumulation in the SN demonstrated that L-DOPA-induced dyskinesia is related to pathological process also occurring in the DAergic cell bodies. We propose that glial cells alterations in the rats presenting LID might reflect a neuroinflammatory reaction underlying neural mechanisms of dyskinesia.

Disclosures: E. Del Bel: None. **M.-.-.-. Bortolanza:** None. **R. Kwiatkoski:** None. **F.E. Padovan-Neto:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.23/S4

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

Support: Defense Threat Reduction Agency (DTRA)

Title: Decreased neutrophil effectiveness and recruitment grants neuroprotection in knockout mice following soman-induced seizure

Authors: *K. LAITIPAYA[1], J. F. IRWIN[1], J. CHANDLER[1], L. SHUMWAY[1], T. FERRARA-BOWENS[1], M. D. WEGNER[2], E. A. JOHNSON[1];
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Abstract: Severe neuropathology and behavioral impairment are a result of prolonged status epilepticus (SE) caused by organophosphorus nerve agents such as soman (GD). GD, a potent acetylcholinesterase inhibitor, causes prominent cell death in the hippocampus, thalamus and piriform cortex, leading to the activation of the neuroinflammatory cascade. Neuroinflammation can exacerbate tissue injury or promote healing, depending on the intricate interaction of multiple cells, inflammatory factors and receptors as the injury progresses, a fact that has complicated the development of effective neuroprotective therapies for CNS injury models such as SE. Neutrophils are important in propagating the inflammatory cascade, can help resolve damage through many inflammation -related pathways and are one of first subset of leukocytes to respond to brain injury following seizurogenic exposure to GD. In scenarios where extensive damage occurs, neutrophils may become overactive and further exacerbate the injury. In this study, knockout mice missing key recruitment/activation proteins such as intracellular adhesion molecule 1 (ICAM-1), leukotriene B_4 Receptor (BLTR), E-selectin, and neutrophil cytosolic factor 1 (NCF1) were used to determine the role of neutrophils in the progression of acute neuropathology following GD induced seizures. Results indicate that reducing the effectiveness of neutrophil recruitment/activation does attenuate acute GD-induced brain damage. These data suggest that limiting leukocyte activation, in particular neutrophils, may be a viable target for neuroprotection and behavioral recovery. Further studies will investigate the more chronic effects of attenuated neutrophil activity on injury development, resolution and recovery.

Disclosures: K. Laitipaya: None. **J.F. Irwin:** None. **J. Chandler:** None. **L. Shumway:** None. **T. Ferrara-Bowens:** None. **M.D. Wegner:** None. **E.A. Johnson:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.24/S5

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

Title: Transcriptome data from multiple cell types isolated from adult murine CNS tissue reveals cell type specific response to CNS insults

Authors: *K. SRINIVASAN, B. A. FRIEDMAN, D. HANSEN;
Genentech Inc, South San Francisco, CA

Abstract: Microglia, the primary immune cells of the brain, play a key role in monitoring the brain for any toxic insults that may result in neuronal damage and cell death. Upon sensing any noxious stimuli, microglia respond robustly by secreting inflammatory cytokines and by phagocytosing debris to maintain an optimal environment for neuronal function. Inflammatory signaling in the brain plays a vital role in the pathology of several neurodegenerative diseases, although it is not clear if the inflammatory response is beneficial. In order to fully understand the biology of neuroinflammation, it is critical to tease apart the gene expression changes in neurons and other cell types including microglia, astrocytes and endothelial cells. Here, we present a method to acutely isolate multiple cell types from a single, adult mouse brain without employing any transgenic reporters for the isolation procedure. Using RNA sequencing data from these isolated cell types, we demonstrate the feasibility of this technique in an endotoxemia model and report that the brain's response to endotoxemia is highly cell type specific and that astrocytes require TNFR1 for this response. We next applied this technique to a mouse model of Alzheimer's disease. Transcriptomic analysis of individual cell types revealed hundreds of differentially expressed genes not observed using whole tissue RNA. For example, microglial cells from diseased brains had altered expression of genes involved in Wnt signaling and lipoprotein metabolism. We were also able to detect genes that were downregulated in microglia. These changes are typically difficult to detect in diseased brains due to increased microgliosis, which masks the decreased levels of gene expression. Applying this method to additional models and patient tissues has the potential to transform our understanding of the role of aberrant gene expression in neurological disease or injury.

Disclosures: K. Srinivasan: None. **B.A. Friedman:** None. **D. Hansen:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.25/S6

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

Support: Defense Threat Reduction Agency (DTRA)

Title: Inhibition at various locations within the TNF signaling pathway after soman-induced seizures in mice

Authors: *J. K. CHANDLER, T. M. FERRARA-BOWENS, J. F. IRWIN, K. LAITIPAYA, L. J. SHUMWAY, M. D. WEGNER, E. A. JOHNSON;
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Abstract: Exposure to organophosphorus compounds such as soman (GD), a potent acetylcholinesterase inhibitor and chemical warfare agent (CWA), results in prolonged status epilepticus (SE) and severe neuropathology in the hippocampus, thalamus, and piriform cortex. Exposure to GD and similar CWAs has been shown to cause neuroinflammation, which exacerbates damage, reduces cognitive abilities, and decreases overall outcomes. TNF signaling, a prominent pro-inflammatory pathway, appears to exacerbate tissue damage in multiple CNS injury models. Results of previous studies indicate that the loss of normal TNFR signaling does afford neuroprotection in various brain regions and can alter seizure onset times and mortality rates. This study investigated the effects of TNFα, a ligand in the pathway, 24 hours after acute exposure to GD, in comparison to the inhibition of TNFR signaling. Multiple TNF pathway knockout mouse strains were exposed to GD, and changes in acute neurodegeneration, mortality, seizure onset and other physiological variables were compared to those background matched wild-type mice. These results, along with neuroinflammation profiles from the mouse model, will further guide the selection of pathway-specific drugs for testing as well as provide an extended therapeutic window for conventional treatments. The views expressed in this talk are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. These studies were funded by the Defense Threat Reduction Agency (DTRA).

Disclosures: **J.K. Chandler:** None. **T.M. Ferrara-Bowens:** None. **J.F. Irwin:** None. **K. Laitipaya:** None. **L.J. Shumway:** None. **M.D. Wegner:** None. **E.A. Johnson:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.26/S7

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

Title: Depressed basal neuronal activity in a genetic model of type-1 diabetes is correlated with proinflammatory secretion of HMBG1

Authors: *J. S. THINSCHMIDT[1], M. FEBO[2], L. M. COLON PEREZ[2], S. CABALLERO[1], M. B. GRANT[3];
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Abstract: We recently found indicators of hypothalamic inflammation and neurodegeneration linked to the loss of neuroprotective factors including insulin-like growth factor (IGF-1) and IGF binding protein-2 (IGFBP-3) in mice made diabetic using streptozotocin. In the current work, a genetic model of type-1 diabetes (Ins2Akita mouse) was used to evaluate changes in neuronal activity and concomitant changes in the proinflammatory mediator high-mobility group box-1 (HMBG1). We found basal hypothalamic neuronal activity as measured by manganese-enhanced magnetic resonance imaging (MEMRI) was significantly decreased in 8 month old, but not 2 month old Ins2Akita diabetic mice compared to controls. In tissue from the same animals we evaluated the expression of HMBG1 using immunohistochemistry and confocal microscopy. We found decreased nuclear HMBG1 colocalization in the paraventricular nucleus of the hypothalamus (PVN) in 8 month old, but not 2 month old diabetic animals indicating nuclear release of the protein consistent with an inflammatory state. Adjacent thalamic regions showed little change in HMBG1 colocalization and neuronal activity as a result of diabetes. This work extends our previous findings demonstrating changes consistent with hypothalamic neuroinflammation in STZ treated animals, and shows active inflammatory processes are correlated with changes in basal hypothalamic neuronal activity in Ins2Akita mice.

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Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

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Program#/Poster#: 74.27/S8

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

Support: CIHR

Title: Role of Toll like receptors in neurogenesis

Authors: *S. KRISHNASAMY, S. THAMMISETTY, Y. WENG, M. LALANCETTE-HÉBERT, J. KRIZ;
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Abstract: Aim: The generation of new neurons in the adult brain is mostly associated with two regions, the sub ventricular zone of the lateral ventricles (SVZ) and the sub granular zone of the hippocampal dentate gyrus (SGZ). TLR2 is one of the most prevalent Toll-like receptors in the central nervous system (CNS). In addition to immune cells in the brain, neurons also express TLRs. TLR2 expression was detected on cells that co-express the early neuronal marker, doublecortin (DCX). The finding that TLR2 is expressed in the adult neurogenic niches prompted us to investigate whether it plays a role in neurogenesis in chronic neurodegenerative disorders. In this study we used a TDP43 mice, a valid animal model of Fronto Temporal Dementia (FTLD) to investigate the role of TLRs in neurogenesis. . Objective: The functional plasticity of CNS, including neurogenesis depends on innate and adaptive immunity. We have investigated the role of TLR2 in neurogenesis in the acute and chronic inflammation. Results: Immunofluorescence analysis of nestin, GFAP and DCX expression in SVZ and DG showed that attenuation of neurogenesis in the TL2 KO mice before and after MCAO. *In vivo* imaging of TLR2 expression study reveals that there is increased expression of TLR2 observed in TDP43 mice compared with their wild type mice. Western blot analysis of TL2 showed that there is higher expression in young TDP43 mice. Conclusion: Attenuation of neurogenesis in TLR2 KO mice reveals the impact of TLRs in neurogenesis. Longitudinal *in vivo* imaging results showed that there is increased expression of TLR2 in the young and old transgenic mice compared to the wild type mice.Induction of LPS produced an over expression of TLR2 and cytokines in the transgenic mice.Immunofluorescence analysis of neural progenitors showed that there is significant increase in the both neurogenic regions pre and post symptomatic conditions.

Disclosures: S. Krishnasamy: None. **S. Thammisetty:** None. **Y. Weng:** None. **M. Lalancette-Hébert:** None. **J. Kriz:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.28/S9

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

Support: Italian Ministry for Foreign Affairs Grant PGR00151

Nando Peretti Foundation

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Title: Dysregulation of the astrocytic S100B/RAGE system in ALS models

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Abstract: Astrocytic dysfunction is believed to play a crucial role in ALS neuroinflammatory processes. Most of the toxic astrocytic effects involve intracellular calcium. S100B is a Ca^{2+}-binding protein particularly abundant in astrocytes, behaving as a neuroinflammatory mediator as it is secreted at high concentration by astrocytes under pathological conditions, displaying paracrine toxicity by binding to RAGE. During ALS progression S100B increases in patient astrocytes and, in a rat model of the disease, S100B is augmented in “aberrant astrocytes”, characterized by their neurotoxic potential. The induction of S100B in astrocytes, its release and its interaction with RAGE in motor neurons could represent an hazardous mechanism that takes place during ALS. Main objectives of this work were to investigate 1) if the expression of S100B protein and RAGE change during the course of the disease in rodent models of ALS; 2) if the expression of mutant SOD1 protein per se is sufficient to modify S100B levels in astrocytic cultures. We observed that S100B levels and localization are modulated in the spinal cord and in the brain cortex of rat and mouse models of ALS. We also demonstrated a differential expression of RAGE subunits in SOD1-G93A-derived CNS tissues. Moreover, we induced overexpression of mutant SOD1 in an astrocytic cell line, and observed that the intracellular levels and even the release of S100B increase in these cells. However, overexpression of mutant SOD1 is not enough to induce a differential expression of RAGE in astrocytic culture. Thus, the mere expression of mutant SOD1 interferes with the physiological expression of S100B and with its release at least in cell culture, while the dysregulation of RAGE, which in our conditions is found only in tissues, might be a phenomenon requiring a more complex interplay between different cell types and pathways, or merely involving other cell types different from astrocytes. Overall, these data suggest that S100B may be a toxic mediator released by astrocytes in the ALS-linked neuroinflammatory process, and that its receptor RAGE might be involved in this pathway. This

possibility might even have translational consequences, indicating S100B as a possible candidate to be a therapeutic target for ALS-linked neuroinflammatory processes.

Disclosures: **F. Michetti:** None. **C. Donno:** None. **P. Andjus:** None. **N. D'ambrosi:** None.

Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 74.29/S10

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

Title: Blood-derived macrophages do not contribute to the inflammatory response after cranial irradiation

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Abstract: Background: Cranial irradiation (IR) induces loss of neural stem and progenitor cells and is followed by progressive cognitive deterioration in both young and adult patients. Earlier studies have suggested that neuroinflammation contributes to the development of adverse effects, including a shift from neurogenesis to gliogenesis. Given the difficulties distinguishing resident microglia from macrophages derived from blood-borne, peripheral monocytes, the relative contribution after cranial IR of these two evolutionarily different groups of macrophages and antigen-presenting cells is not known. **Methods:** $CX3CR1^{GFP/+}CCR2^{RFP/+}$ mice were used to distinguish resident microglia ($CX3CR1^{+}$; GFP-labeled) from monocyte-derived macrophages ($CCR2^{+}$; RFP-labeled). CCL2 expression and infiltrating monocytes in hippocampus and cerebellum and microglial response in the hippocampus were determined by ELISA and immunofluorescent staining from 6 h through 1 month following a single dose of 8 Gy IR on juvenile (PND 10) or adult (PND 90) brain. **Results:** Our results demonstrate that CCL2 was increased by 360 % in PND 10 and 120 % in PND 90 mice at 6 h and remained elevated for at least 1 month after IR. No peripheral monocyte-derived macrophages could be detected in the hippocampus at any time point investigated (6 h, 1 day, 1 week, 1 month) after IR, neither in juvenile nor in adult mice. In the juvenile brain, the microglia density in the granule cell layer (GCL, including the subgranular zone (SGZ)) increased 6 h, peaked at 1 day, and decreased

thereafter back to the baseline 1 month after IR. In the adult brain, however, the microglia density peaked already 6 h after IR, returned to baseline within 1 day and decreased thereafter. The microglia increase in the juvenile GCL was probably attributed to both proliferation and migration from the molecular layer. Starting from 6 h after IR, microglia in SGZ exhibited amoeboid morphology and expressed the marker CD68. This activation lasted for 1 week in the juvenile brain but only 1 day in the adult brain, indicating a more transient and less pronounced microglia activation in the adult brain after IR. **Conclusions:** In summary, we show here for the first time that peripheral monocyte-derived macrophages do not appear to contribute to the inflammatory response in the hippocampus after IR, neither in the juvenile nor in the adult brain. We also show that the microglia response is more pronounced and protracted in the juvenile brain, and that the inflammation, as judged by CCL2 levels, appears chronic, lasting at least one month after IR.

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Poster

074. Neuroinflammation: Endogenous and Exogenous Modulation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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

Title: Time-specific changes in kainic acid-induced mesial temporal lobe epilepsy: transcriptomic and immunohistochemical evaluation

Authors: *M. E. HAMBY, S. M. FALLON, J. A. TAMM, J. SERRATS, M. J. DENBLEYKER, A. ABDOURAHMAN, G. TERRY, R. B. NELSON, B. M. CAMPBELL, P. D. WES, T. MÖLLER, N. BREYSSE;
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Abstract: Mesial Temporal Lobe Epilepsy (MTLE) is a chronic disease characterized by recurrent unprovoked partial seizures. Current treatments lack long-term efficacy and typically act only to ameliorate the symptomology, rather than to modify the course of the disease. Chronic kainic acid (KA)-induced models of MTLE mimic prominent histopathological and electroencephalographic features of human MTLE, including hippocampal sclerosis, CA1 neuronal loss, astrogliosis, microgliosis, and hippocampal discharges. Here we examined the transcriptional profiles of the hippocampus of KA- vs sham-injected mice from epileptogenesis to the stabilization of spontaneous recurring seizures. KA (1 nmol) or saline (sham) was injected

unilaterally into the dorsal hippocampus of 12 week old C57BL/6J mice, and the ipsilateral (IL) and contralateral hippocampi were isolated for whole-genome expression profiling (Illumina microarray) or immunohistochemistry 7, 28 and 60 d later. Differential gene expression analysis of the IL hippocampus indicated that 1349, 484, and 439 genes were significantly (fdr<0.05) altered in KA compared to sham at 7, 28 and 60 d, respectively. 108 genes were altered at all time-points assessed, while others were altered in a time-dependent manner, indicative of distinct hippocampal gene expression profiles across the course of disease. Ingenuity Pathway Analysis revealed that specific immune cell and inflammatory signaling pathways were upregulated at 7 and 28 d, in support of a role of neuroinflammation in the disease etiology. The top canonical pathways profile at 60 d was quite distinct from that at 7 and 28 d, although certain inflammatory pathways were still prevalent, indicative of pathways involved in the maintenance of the disease. Consistent with a role of neuroinflammation in MTLE, immunohistochemical analyses demonstrated time-dependent changes in both the intensity of immunoreactivity and number of Iba1- and GFAP-positive cells. To reveal gene networks not apparent with differential expression data alone, weighted gene co-expression analysis (WGCNA) was performed. A gene network that highly associated with 7 d IL-KA (but not 28 and 60 d) was identified, which may point to changes involved in the process of epileptogenesis. Another gene network revealed a group of genes highly associated with changes occurring at 28 and 60 d, but not 7 d, which may point to molecules involved in the generation of spontaneous recurring seizures. Collectively, results indicate that specific pathways, including many associated with neuroinflammation, are activated in a time-dependent manner in MTLE.

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Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: E.05. Stress and the Brain

Support: NIH MH090297

Title: NPY and CRF induce bi-directional, long-term morphological effects on BLA pyramidal cells as structural correlates of stress resilience and vulnerability

Authors: *S. D. MICHAELSON[1], A. P. MIRANDA[1], H. SILVEIRA VILLARROEL[1], A. MCKINTY[1], K. EPPLER[1], J. WANG[1], Y. QIU[1], J. H. URBAN[2], W. F. COLMERS[1]; [1]Univ. of Alberta, Edmonton, AB, Canada; [2]Physiol., Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL

Abstract: Many brain regions respond to stress with the basolateral amygdala (BLA) regulating emotional processing. Glutamatergic pyramidal cells are the major output neurons of the BLA and mediate behavioral stress responses. These cells respond to stress with dendritic hypertrophy and increased spine densities. Several endogenous neuromodulators alter BLA output cell activity, including the anxiolytic neuropeptide Y (NPY) and the anxiogenic corticotrophin releasing factor (CRF). Daily (5 x d) injections of NPY or urocortin (CRFR1 agonist), induce a persistent state of stress resilience or vulnerability, respectively. While the acute anxiolytic effect of NPY in the amygdala is mediated in part by Y1 receptors, the NPY receptor inducing stress resilience is unknown. We hypothesized that chronic CRF treatment would recapitulate the effects of stress observed on BLA pyramidal cells and NPY treatment would result in the opposite effect, namely, dendritic retraction. We tested this in male rats *in vivo* and using a novel organotypic slice culture preparation of BLA (BLA-OTC) we recently developed. BLA-OTCs (6 w postnatal equivalent age) were treated with NPY, NPYR subtype-selective agonists or CRF for 5 days. Electrophysiological and morphological changes in neurobiotin-filled neurons were analyzed 7-14 days after peptide treatment. In parallel, NPY or a Y1R agonist were injected daily (5 x d) into the BLA of 7 week-old rats; social interaction (SI) was assessed and similar recordings were made in acute BLA slices 4 weeks after the first injection. NPY increased SI time as expected ($\geq$ 4 weeks). Intra-BLA injections of the Y1R ligand increased SI 30 min after injection but did not affect SI long-term. BLA pyramidal cell dendrites were retracted in NPY-treated, but not Y1 agonist-treated rats. In the BLA-OTC, NPY, but not the Y1 agonist, decreased pyramidal cell dendritic length as determined by Sholl analysis, while CRF produced dendritic hypertrophy similar to that seen with stress. Application of the Y5 receptor ligand, (possibly anxiolytic *in vivo*) also caused dendritic retraction, while activation of Y2 receptors, which causes acute anxiety *in vivo*, caused dendritic hypertrophy similar to CRF. The BLA-OTC preparation thus may predict structural changes that accompany stress resilience and vulnerability. NPY and CRF cause opposite effects on BLA dendrites correlating with behavioral changes seen with peptide treatment *in vivo*. The contribution of NPY receptor subtypes to the generation of these changes will be further examined using Y5 and Y2 receptor-selective agonists in rats to determine if they induce long-term behavioral stress resilience or vulnerability.

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Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.02/S13

Topic: E.05. Stress and the Brain

Support: NIH Grant MH090297

Title: Characterization of Y2 receptor-expressing cells in the basolateral amygdala

Authors: M. G. DEJOSEPH[1], J. P. MACKAY[2], W. F. COLMERS[2], *J. H. URBAN[1];
[1]Physiol. & Biophysics, Chicago Med. Sch/Rosalind Franklin Univ. Med. & Sci., North Chicago, IL; [2]Pharmacol., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Neuropeptide Y (NPY) plays an important role in the buffering of anxiety responses, predominantly through activation of Y1 and Y5 receptor subtypes in the basolateral amygdala (BLA). Conversely, activation of Y2 receptors in this same region has been suggested to be anxiogenic or promote stress-related behaviors. The mechanism and circuitry underlying these diametrically opposed effects of NPY Y2 receptor activation on BLA activity has yet to be elucidated. In this context, we have studied a novel transgenic mouse line (NPY2R-cre x Rosa26-CAG-tdTomato = Y2R-tdTomato) to aid in the characterization of Y2R-related circuitry in the BLA. To determine the neurochemical identity of Y2R-tdTomato-expressing cells in the BLA, brain sections through the amygdala ofY2R-tdTomato mice were incubated with combinations of antibodies to identify glutamatergic pyramidal cells (CaMKII) or interneurons [NPY, somatostatin (SOM), parvalbumin]. tdTomato (putative Y2R)-expressing cell bodies were found throughout the rostral-caudal aspect of the BLA. In the anterior-most BLA, clusters of tdTomato-expressing cells were present within the medial aspect of the nucleus. A majority (>50%; cell counts pending) of these cells were also CaMKII-immunopositive, suggesting that the Y2R may regulate the activity of glutamatergic projection neurons, as occurs in the hippocampus (El Bahh et al, Eur. J. Neuroscience, 22: 1417-1430 (2005). The vast literature indicates that the Y2R may also function as a presynaptic autoreceptor on NPY neurons, which comprise a population of BLA interneurons. All double labeled NPY/SOM-immunoreactive neurons in the BLA and surrounding regions also expressed tdTomato, further reinforcing the role of the Y2R in regulating the activity of NPY neurons. By contrast, parvalbumin-immunoreactive neurons were devoid of tdTomato signal. The presence of Y2R expression in NPY interneurons as well as subclasses of glutamatergic provides a complex and testable circuit to examine the effects of Y2 receptor signaling on the generation of acute as well as persistent anxiety responses.

Disclosures: **M.G. DeJoseph:** None. **J.P. Mackay:** None. **W.F. Colmers:** None. **J.H. Urban:** None.

Poster

075. Cells and Circuits of Stress

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Topic: E.05. Stress and the Brain

Support: NIH Grant MH090297

Title: HCN1 channel expression in the BLA and anxiety-related behavior

Authors: ***M. BOMPOLAKI**[1], W. F. COLMERS[3], J. H. URBAN[2];
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[3]Pharmacol., Univ. of Alberta, Alberta, AB, Canada

Abstract: The activity of pyramidal neurons in the basolateral nucleus of the amygdala (BLA) is predictive and consistent with the expression of anxiolytic- and anxiogenic-like behaviors in rats. Intra-BLA injections of Neuropeptide Y (NPY) reduce BLA output and are anxiolytic, whereas intra-BLA injections of Corticotropin Releasing Factor (CRF) increase BLA output and are anxiogenic. Previous studies from our laboratories demonstrated that both NPY and CRF act at the neuronal level by mediating the inhibition or enhancement, respectively, of the hyperpolarization activated, depolarizing conductance, the H current, carried by HCN channels (Geisbrecht et al., 2010). These studies, together with anatomical studies, indicated that it is likely that the actions of NPY and CRF result from bidirectional modulation of pyramidal cell HCN1 subunits as their final common pathway. Furthermore, repeated intra-BLA injections of NPY produce prolonged (weeks to months) behavioral stress resilience, as they markedly increase the time treated rats spend interacting in the social interaction (SI) test (Sajdyk et al., 2008). We now have data suggesting HCN1 expression is decreased in the BLA in NPY-induced stress resilience. Therefore, we hypothesize that the expression level of HCN1 in the BLA is important in mediating stress-related behavior. To test this, we used a lentivirus expressing shRNA targeting mRNA for HCN1 to selectively reduce the expression of HCN1 in the BLA of male rats and stress-related behavior was assessed. Animals received injections (2 μL per side; 109 titer units/mL concentration of virus) directly into the BLA of lentivirus expressing either shHCN1 or a scrambled (non-coding) shHCN1 sequence, or vehicle (saline) alone. Four weeks later, immunohistochemistry revealed significant reductions in HCN1 protein levels within the infected region. Social interaction was studied in all groups of injected animals at several times

following the injections. As in chronically NPY-treated animals, SI times were increased in animals that received shHCN1-injections when compared to the other two control groups. All cohorts of animals were also tested in the elevated plus maze (EPM) test. Interestingly, knockdown of BLA HCN1 expression did not have an effect on the performance of these rats on the EPM. These data reinforce our hypothesis that the H current plays a critical role in regulating BLA output, which in turn has significant consequences for regulation of behavioral responses to stress and on the expression of anxiety-related behaviors.

Disclosures: M. Bompolaki: None. **W.F. Colmers:** None. **J.H. Urban:** None.

Poster

075. Cells and Circuits of Stress

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Topic: E.05. Stress and the Brain

Support: NIH Grant MH-072908

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Title: Muscarinic receptors modulate intrinsic GABAergic transmission in the bed nucleus of stria terminalis

Authors: *J. GUO, Y. YANG, D. G. RAINNIE;
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Abstract: The bed nucleus of stria terminalis (BNST) is an important relay in the circuits regulating emotion. Neurons of the BNST are primarily GABAergic, and are heavily interconnected. Hence, modulation of intrinsic connections within the BNST most likely determines the output of BNST subnuclei to downstream targets. Using a combination of optogenetics and *in vitro* patch clamp recording, we examined intrinsic connections between BNST neurons, and its modulation by acetylcholine (Ach). Application of carbachol (CCh) depolarized BNST neurons, irrespective of cell type, and increased the rate of action potential firing, an effect that was blocked by the muscarinic receptor antagonist, atropine or the M1 receptor antagonist, pirenzepine. Due to the marked intrinsic connectivity, an increased rate of firing in BNST neurons would be predicted to increase local GABA release. As expected, CCh application dramatically increased both the frequency and amplitude of spontaneous IPSCs. Moreover, the CCh effect was blocked by atropine or the sodium channel blocker, tetrodotoxin;

but not by the AMPA receptor antagonist DNQX. Together these data suggest that postsynaptic activation of muscarinic receptors in BNST neurons results in enhanced local inhibitory tone. Paradoxically, CCh was also observed to reduce the frequency but not the amplitude of miniature IPSCs, suggesting that CCh can also inhibit presynaptic GABA release. CCh also decreased the amplitude of stimulation-evoked IPSCs, and increased the paired-pulse-ratio. However, electrical stimulation cannot differentiate between intrinsic GABAergic connections and extrinsic GABAergic inputs. Hence, we used optogenetics to selectively activate intrinsic GABAergic connections by locally injecting an AAV Hsyn-ChR2-eYFP construct into the anterior BNST. As expected light activation of BNST neurons evoked monosynaptic IPSCs that had a reversal potential close to chloride equilibrium potential, and were blocked by GABAA receptor antagonist, SR95531. Light-evoked IPSCs were seen in all three types of BNST neuron. Significantly, the light-evoked IPSCs were attenuated by muscarine, which also increased the coefficient of variation of the IPSCs, suggesting a presynaptic locus of action. Moreover, the effect of muscarine was blocked by atropine. Together these data suggesting that presynaptic muscarinic receptor can modulate intrinsic GABAergic transmission. Experiments are in progress to determine the effects of Ach on extrinsic GABAergic transmission. Differential modulation of intrinsic or extrinsic GABAergic transmission may have important implications for signal processing in the BNST.

Disclosures: J. Guo: None. **Y. Yang:** None. **D.G. Rainnie:** None.

Poster

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Title: Chronic stress modulates synaptic strength in the bed nucleus of the stria terminalis

Authors: *S. E. DEWITT[1], A. MENIGOZ[2], J. GUO[2], D. G. RAINNIE[2];
[1]Behavioral Neurosci. and Psychiatric Disorders, [2]Emory Univ., Atlanta, GA

Abstract: Chronic stress causes a reduction in striatal-enriched protein tyrosine phosphatase (STEP) and an increase in synaptic plasticity in Type III, putative CRF neurons in the dorsal bed nucleus of the stria terminalis (dBNST). STEP opposes synaptic strengthening by inhibiting the activation of ERK and reducing AMPA receptor (AMPAR) insertion in the membrane. The loss of STEP in Type III cells may cause an increase in the ratio of AMPA to NMDA receptors indicative of synaptic strengthening. Although most AMPARs are impermeable to calcium, AMPARs lacking the GluR2 subunit pass calcium and activate the ERK pathway. Chronic stress modulates CP-AMPARs in the BNST of mice however it is unknown if this is differentially affected by cell type. We have begun to determine how chronic stress affects the relative contribution of AMPA, CP-AMPA, and NMDA receptors to the evoked excitatory post-synaptic current (eEPSC) in Type I, II, and III neurons in the dBNST. To this end, we used a 7-day unpredictable shock stress paradigm (USS) on rats. Five days after stress, the AMPA-NMDA ratio (AMPA:NMDA) and CP-AMPA-AMPA ratio were analyzed in the dBNST. Single cell RT-PCR (scRT-PCR) was used to examine the effects of stress on the relative expression of AMPA and NMDA subunits as well as genes involved in the ERK signaling cascade. Our preliminary results have shown a trend towards an increase in the AMPA:NMDA in stress rats (1.5 ± 0.2) compared to controls (1.1 ± 0.2; $p=0.12$). In addition, 1-naphthylacetyl spermine trihydrochloride (NASPM), a CP-AMPA antagonist, reduced the amplitude of the eEPSC at a slower rate (peak response 30% reduction in stress and 44% in controls; interaction between time and stress [$F(19,228) = 1.644$, $P < .05$]) and washed out faster in stressed rats (interaction [$F(21,168) = 1.623$, $P < .05$]. These data are indicative of a reduction in the contribution of CP-AMPARs to the eEPSC in rats exposed to the USS. The preliminary data indicates the reduction in CP-AMPA current specifically occurs in non-Type III cells. In this model, after chronic stress Type III neurons decrease STEP expression leading to an increase in AMPA and CP-AMPA membrane insertion while non-Type III neurons show a reduction in the relative contribution of CP-AMPARs. These differential effects in Type III and non-Type III cells cause an increase of excitability in Type III, putative CRF neurons, while decreasing the excitability of non-Type III cells thereby increasing the anxiogenic output of the dBNST. In a parallel set of experiments, we are using scRT-PCR to examine the expression of GluR1 and GluN1 mRNA in the different cell types to determine if USS induces a cell type specific alteration in receptor expression.

Disclosures: **S.E. Dewitt:** None. **A. Menigoz:** None. **J. Guo:** None. **D.G. Rainnie:** None.

Poster

075. Cells and Circuits of Stress

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Title: Dopamine (D1R) antagonist suppresses corticolimbic oscillations and fear learning

Authors: *T. E. MADSEN[1], C.-C. HSU[1], C. N. COLLINS[2], J. S. PITTS[3], D. G. RAINNIE[1]; [1]Behavioral Neurosci. and Psychiatric Disorders, Emory Univ., Atlanta, GA; [2]GRU Med. Col. of Georgia, Augusta, GA; [3]Agnes Scott Col., Decatur, GA

Abstract: Activation of dopamine D1 family receptors in the basolateral amygdala is required for successful acquisition of conditioned fear. D1Rs are also critical for the *in vitro* generation of long-term potentiation (LTP), often assumed to be the cellular substrate of learning. Furthermore, dopamine modulates oscillatory activity in the amygdala, which facilitates communication with other limbic brain regions. Delta/theta and gamma oscillations in the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC) have recently been associated with fear learning and expression. We hypothesized that the blockade of fear learning by the D1R antagonist is mediated by its suppression of corticolimbic oscillations. Adult, male, Sprague-Dawley rats were first trained to nosepoke for a food reward, in order to establish a baseline of activity against which to assess freezing as a measure of fear. They were then implanted with microwire recording arrays in the BLA and mPFC. SCH23390 (a selective D1R antagonist) or saline was administered by I.P. injection immediately prior to tone habituation and classical auditory fear conditioning. Fear recall was tested two days later, in the absence of drug. Neuronal spikes and local field potentials (LFPs) were simultaneously recorded in both regions throughout fear acquisition and extinction. Rats that received the D1R antagonist demonstrated significantly less fear recall (freezing and nosepoke suppression) than saline treated rats, despite equivalent responses to acute shock during conditioning. In control rats, the BLA and mPFC displayed significantly increased power, and coherence between regions, in a sharply tuned delta/theta (2-6 Hz) band during successful fear acquisition and recall, as compared to baseline (before habituation tones). Throughout fear acquisition, the mid-gamma (45-60 Hz) range was significantly elevated over baseline in terms of mPFC power, BLA power, and coherence between the two regions. After the shock, there were dramatic increases in high gamma (60-90 Hz) power for the mPFC and in low gamma (30-45 Hz) power for the BLA, as well as mid-gamma within and between both regions. All of these fear-related changes in oscillatory activity were reduced in D1R antagonist treated rats, to the extent that no tone-triggered changes were apparent upon fear recall testing. These results demonstrate a strong correlation between dopamine D1R activation, corticolimbic oscillations, and fear learning, such that we believe D1Rs critically modulate corticolimbic oscillations, which are necessary substrates of fear learning.

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Poster

075. Cells and Circuits of Stress

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Topic: E.05. Stress and the Brain

Support: NIH T32GM008605-19

Title: High frequency stimulation changes *in vitro* basolateral amygdala excitability

Authors: *B. O'FLAHERTY[1], D. RAINNIE[2];
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Abstract: Deep brain stimulation (DBS) of the insular cortex at 130 HZ is an effective treatment of major depressive disorder (MDD) in patients that are unresponsive to traditional MDD therapies such as SSRIs. However, it is currently unknown why DBS is an effective MDD treatment. To date, no one has shown how 130 Hz stimulation affects neuronal activity in limbic areas known to be dysregulated in MDD. For example, the basolateral amygdala (BLA; a key mood area that is hyperactive in MDD patients) receives dense synaptic projections from the infralimbic cortex, and may be a key region influenced by DBS in humans. We thus hypothesize that 130 Hz stimulation of synaptic inputs to the rat BLA would reduce BLA principal neuron excitability. To test this hypothesis, we used *in vitro* whole-cell patch clamp recordings from BLA principal neurons in coronal slices, in conjunction with 130 Hz extracellular stimulation of the external capsule (EC). The EC was stimulated for periods of 2, 4, 6, and 8 minutes. Input resistance, spike threshold, spontaneous synaptic activity frequency / amplitude, and evoked synaptic activity amplitude were measured before and immediately after 130 Hz stimulation. No change in membrane resistance or spike threshold was seen in BLA principal neurons after 130 Hz stimulation. However, we observed a marked decrease in evoked synaptic activity amplitude after 130 Hz stimulation. We interpret the reduced evoked synaptic response as a depletion of readily-releasable synaptic vesicles at cortico-amygdala and thalamo-amygdala synapses. As hyperactivity of the BLA has been implicated in MDD, a reduction in synaptic input into the BLA principal neurons could reduce BLA activity and restore normal functioning. Understanding how 130 Hz stimulation affects the BLA is vital for understanding the clinical efficacy, and to develop more effective and less invasive treatments for treatment-resistant MDD.

Disclosures: **B. O'Flaherty:** None. **D. Rainnie:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.08/S19

Topic: E.05. Stress and the Brain

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Vanderbilt DRTC

NARSAD Distinguished Investigator Grant: 21809

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NIH grant AA022937

Title: Mapping the excitatory afferents onto corticotropin releasing factor neurons in the bed nucleus of the stria terminalis

Authors: ***T. FETTERLY**[1,2,3,4], K. M. HOLLERAN[2,3,4], E. K. AWAD[1], Y. SILBERMAN[1,3,4], D. G. WINDER[1,2,3,4];
[1]Mol. Phys & Biophys, Vanderbilt Univ. Med. Ctr., Nashville, TN; [2]Brain Inst., [3]Neurosci. Program in Substance Abuse, [4]Kennedy Ctr., Vanderbilt Univ., Nashville, TN

Abstract: Corticotropin releasing factor (CRF) is involved in the regulation of many behaviors, including feeding, substance abuse and withdrawal, and affective states. Studies, including our own, have implicated excitation of CRF neurons within the bed nucleus of the stria terminalis (BNST) as being of importance in interactions between stress and drugs of abuse (such as cocaine and alcohol). One challenge in studying CRF neurons within the BNST is the complex circuitry and heterogeneity of the brain region. We are utilizing optogenetic and genetically encodable reporter strategies to map excitatory afferents that synapse directly on CRF neurons within the BNST. We have focused on glutamatergic inputs based on two observations. First, qRT-PCR data shows a decrease in BNST CRF mRNA levels in C57Bl/6J mice 24 hours following an injection of ketamine, an N-methyl D-aspartate receptor antagonist. Second, we find that α- and β-adrenergic receptors reciprocally regulate excitatory drive onto BNST CRF neurons and modulate stress-induced fos responses in these cells *in vivo*. Thus, we have stereotaxically injected female CRF-tdtomato reporter mice (strain B6(Cg)-Crhtm1(cre)Zjh/J

crossed with strain B6.Cg-Gt(ROSA)26Sor<tm14(CAG-tdTomato)Hze>/J) with 300 nl of AAV5-CamKIIα-ChR2:YFP into one of three different brain regions with known glutamatergic innervation of the BNST: anterior insular cortex, medial prefrontal cortex (mPFC), or the parabrachial nucleus (PBN). Using whole-cell patch-clamp electrophysiological analysis of excitatory post-synaptic currents (EPSCs) elicited by full field blue light illumination in brain slices containing BNST from tdtomato positive neurons, we have observed EPSCs from mice expressing ChR2 in each of the targeted regions. Curiously, while stimulation of insular (8/8 cells; 4 mice) and PBN (2/2 cells; 1 mouse) projections resulted in light-induced EPSCs in all patched CRF neurons to date, only a small subset of CRF neurons responded to stimulation of the mPFC projection (4/24 cells; 5 mice) projection. One reason for these differences could be the substantial heterogeneity of CRF neurons with the BNST. Using current clamp profiles, our lab and others have shown there are at least three different types of CRF neurons. Data from our lab further exemplifies this heterogeneity by showing that ~24% of BNST CRF neurons are calbindin+ and ~9% of BNST CRF neurons project to the ventral tegmental area. Moving forward, we will work to further understand the role of such divergent afferent circuit connectivity to BNST CRF neurons and how stress and norepinephrine can modulate these glutamatergic inputs.

Disclosures: **T. Fetterly:** None. **K.M. Holleran:** None. **E.K. Awad:** None. **Y. Silberman:** None. **D.G. Winder:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

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Program#/Poster#: 75.09/S20

Topic: E.05. Stress and the Brain

Support: NIH Grant DA019112

NIH Grant DA034428

NIH Grant AA022937

Title: Guanfacine activates crf-negative bnst neurons: interaction with hcn channels?

Authors: ***N. A. HARRIS**[1,2,3,4], S. A. FLAVIN[1,5], E. K. AWAD[1,2], Y. SILBERMAN[1,3,4], D. G. WINDER[1,2,3,4,5];

[2]Mol. Physiol. and Biophysics, [3]Neurosci. Program in Substance Abuse, [4]Kennedy Ctr., [5]Vanderbilt Brain Inst., [1]Vanderbilt Univ., Nashville, TN

Abstract: Stress is a major risk factor for relapse to drug-seeking behavior. The bed nucleus of the stria terminalis (BNST) receives an extensive noradrenergic input and has been shown to be critical in stress-induced relapse to drug-seeking. In rodents, systemic or intra-BNST treatment with α2-adrenergic receptor agonists decreases stress-induced reinstatement behaviors. Clinical studies report that guanfacine, an α2A-AR agonist, curbs cravings for a variety of substances of abuse. We have previously shown that α2A-ARs are heavily expressed in the BNST, and that noradrenergic signaling in the region is dramatically altered in α2A-AR knockout mice. Ongoing studies in our lab suggest that competing inhibitory and excitatory actions of this drug in the BNST could reflect carefully coordinated control of divergent neuronal populations. Consistent with this idea, we find that stress increases c-fos expression in both CRF+ and CRF- cells in the BNST, using a CRF-tdtomato reporter mouse line (strain B6(Cg)-Crhtm1(cre)Zjh/J crossed with strain B6.Cg-Gt(ROSA)26Sor/J). Administration of guanfacine prior to the stress suppresses the c-fos response in only CRF+ cells. Moreover, guanfacine alone enhances the c-fos levels in CRF- cells. Our lab has shown inhibitory effects of guanfacine in the BNST on electrically evoked EPSCs and optically evoked EPSCs derived from parabrachial nucleus (PBN) afferents. This is consistent with canonical Gαi signaling and inhibition of CRF+ neurons. In contrast, in Thy1-COP4 transgenic mice (B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J) where ChR2 expression is much reduced in PBN afferents relative to others, bath application of guanfacine enhances optically evoked BNST field potentials. We hypothesize that this novel excitatory effect of Gαi signaling occurs through postsynaptic α2A-ARs in CRF- cells, leading to decreased cAMP-dependent opening of hyperpolarization-activated cyclic nucleotide-gated nonselective cation (HCN) channels. Here we show that bath application of the nonselective HCN inhibitor ZD7288 in *ex vivo* BNST slices from Thy1-COP4 mice enhances optical field potentials by 25%, an effect comparable to that of guanfacine. Furthermore, after systemic guanfacine treatment in a c-fos-eGFP mouse line (B6.Cg-Tg(Fos/EGFP)1-3Brth/J), current clamp profiles of c-fos+ BNST neurons show a significant Ih contribution in 80% of neurons. These converging lines of evidence suggest that excitatory actions of α2A-AR agonism are due to interactions with HCN channels in CRF- BNST neurons. Future studies will elucidate the mechanism of guanfacine enhancement and identify markers of BNST CRF- neurons.

Disclosures: **N.A. Harris:** None. **S.A. Flavin:** None. **E.K. Awad:** None. **Y. Silberman:** None. **D.G. Winder:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.10/T1

Topic: E.05. Stress and the Brain

Title: Association of N-cadherin levels and downstream effectors of Rho-GTPases with dendritic spine loss induced by chronic stress in rat hippocampal neurons

Authors: *J. L. FIEDLER[1], P. CATAÑEDA[2], M. A. MUÑOZ-LLANOS[1], G. GARCÍA-ROJO[1], R. MARQUEZ[1], M. GARCÍA-PÉREZ[1], P. S. ROJAS[1], E. ALIAGA[3];
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Abstract: Chronic stress promotes cognitive impairment and dendritic spine loss in hippocampal neurons. In this animal model of depression, spine loss probably involves a weakening of the interaction between pre- and postsynaptic cell adhesion molecules such as N-cadherin, followed by disruption of cytoskeleton. N-cadherin, in concert with catenin, stabilizes the cytoskeleton through Rho-family GTPases. Via their effector LIM-kinase, RhoA and RAC-GTPases phosphorylate and inhibit cofilin, an actin-depolymerizing molecule, favouring spine growth. Additionally, RhoA through ROCK inactivates myosin phosphatase through phosphorylation of the myosin-binding subunit (MYPT1), producing acto-myosin contraction and probable spine loss. We evaluated whether N-cadherin/β-catenin and Rho-signalling are sensitive to chronic restraint stress. Stressed rats exhibit anhedonia, impaired associative learning and immobility in the forced swimming test and reduction in N-cadherin levels but not β-catenin in the hippocampus. We observed a reduction in the spine number in the apical dendrites of CA1 pyramidal neurons. Although, the stress did not modify the RAC-LIMK-cofilin signalling pathway, we observed an increase phospho-MYPT1 levels, probably mediated by RhoA-ROCK activation. Our findings suggest that a dysregulation of RhoA-ROCK activity by chronic stress could potentially underlie spine loss in hippocampal neurons.

Disclosures: J.L. Fiedler: None. **P. Catañeda:** None. **M.A. Muñoz-Llanos:** None. **G. García-Rojo:** None. **R. Marquez:** None. **M. García-Pérez:** None. **P.S. Rojas:** None. **E. Aliaga:** None.

Poster

075. Cells and Circuits of Stress

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.11/T2

Topic: E.05. Stress and the Brain

Support: CONICYT Grant 21120711

Title: The Rho-Kinase Inhibitor, Fasudil, prevents depressive-like behavior and hippocampal dendritic spine loss promoted by chronic restraint stress in rats

Authors: *G. GARCÍA ROJO, N. VILCHES, A. GARCÍA, F. AGUAYO, J. L. FIEDLER; Univ. De Chile, Santiago, Chile

Abstract: Brain atrophy accompanied by dendritic arbor simplification and reduction in spine density of the hippocampus, a limbic structure implicated in mood disorders, are factors that seems to contribute to the symptoms of depression. It is plausible that these morphological changes imply alterations in the dendritic cytoskeleton dynamic. RhoA is an important regulator of actin dynamic through its effector ROCK. Transfection of hippocampal pyramidal neurons with constitutively active ROCK mutant produces dendritic simplification and dendritic spine loss. We propose that ROCK inhibitor as fasudil (also known as HA-1077), may prevent both the stress-induced depressive like-behavior in rats and the spine density reduction in the hippocampus. Adult male Sprague-Dawley rats were injected i.p. with saline or fasudil (10 mg/kg) starting four days prior restraint stress procedure, which was conducted 2.5 hrs/day during 14 consecutive days. Control animals were injected with saline or fasudil during 18 days. In order to observe the effect of fasudil on depression- like behavior promoted by stress, we carried out the forced swimming test and conditioned avoidance test. Fasudil prevents the stress-induced immobility observed in forced swimming test. In addition, fasudil prevents the stress-induced reduction in conditioned avoidance responses. On the other hand, control animals treated with fasudil showed similar behavioral patterns as control saline animals. Furthermore, Fasudil prevents the stress spine loss in the dorsal hippocampus promoted by chronic stress. Thus we proposed that Fasudil may prevent abnormal behavior promoted by stress probably blocking the ROCK activity with a concomitant prevention of the spine loss.

Disclosures: **G. García Rojo:** None. **N. Vilches:** None. **A. García:** None. **F. Aguayo:** None. **J.L. Fiedler:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.12/T3

Topic: E.05. Stress and the Brain

Support: SBBMCH Grant 2014

Title: Effect of stress on specific microRNAs levels that target genes coding for key proteins involved in spine morphology

Authors: *M. A. GARCÍA PÉREZ[1], M. A. MUÑOZ-LLANOS[1], X. XU[2], J. CIDLOWSKI[2], F. AGUAYO[1], G. GARCÍA-ROJO[1], J. L. FIEDLER[1], A. A. PACHECO[1];
[1]Univ. of Chile, Santiago, Chile; [2]NIEHS, United States, NC

Abstract: MicroRNAs (miRNAs) are small non-coding RNAs that regulate mRNAs translation by matching a seed sequence in the 3'UTR which induces the silencing or degradation of transcripts. MiRNAs are profusely reported in several tissues, cell types and models. They have been postulated as key regulators of relevant processes such as neuroplasticity and brain functions. Recently, we determined the effect of stress on miRNAs levels in the hippocampus. The aims of this study were to (i) verify by RT-qPCR those variations in hippocampus during and after acute and chronic restraint stress sessions (ii) evaluate the putative mRNAs targets relevant for brain function by bioinformatics analyses. Adult male rats were stressed during 0.5 or 2.5 h and sacrificed immediately after restraint session, or 6 and 24 h post stress for acute stress. For chronic stress the rats were stress 2.5 h per day for 14 days and they were sacrificed 24hrs after the last session. We determined that miR-132 and miR-138 did not change in acute stress. Nonetheless, miR-138 showed a significant increase (2 folds) in the chronic stress model. Interestingly, it has been demonstrated that miR-138 has a relevant role in spine morphology and has multiple protein targets relevant in remodeling pathways such as LIMK, ROCK, among others.

Disclosures: M.A. García Pérez: None. **M.A. Muñoz-Llanos:** None. **X. Xu:** None. **J. Cidlowski:** None. **F. Aguayo:** None. **G. García-Rojo:** None. **J.L. Fiedler:** None. **A.A. Pacheco:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.13/T4

Topic: E.05. Stress and the Brain

Title: Relationship between FMRP and MMP9 in an acute restraint stress model in rats: a possible cross-regulation mechanism

Authors: *F. I. AGUAYO, SR[1], P. ROJAS[2], A. A. PACHECO[1], G. J. GARCÍA-ROJO[1], M. GARCÍA-PÉREZ[1], M. MUÑOZ-LLANOS[1], R. MÁRQUEZ[1], J. L. FIEDLER[1];
[1]Facultad De Ciencias Químicas Y Farmacéticas - Uni, Santiago, Chile; [2]Escuela de Química y Farmacia, Univ. Andrés Bello, Santiago, Chile

Abstract: Proteins of Extracellular Matrix (ECM) are important for development and regeneration of nervous system, besides are important in synaptic plasticity. The Matrix Metalloproteinase 9 (MMP9) participates remodeling proteins of ECM, such as: cytokines, growth factors, membrane receptors, adhesion molecules and other proteases. An increase of MMP9 activity has been associated to pathological conditions such as inflammatory diseases. In contrast, an increase in MMP9 activity has been described during LTP, which is considered as a learning model. The increase in MMP9 activity may occur by an altered transport and stability of mRNA, variations in protein level, secretion and post-translation processing. It has been observed in KO mouse for Fragile X Mental Retardation Protein (FMRP) an increase in the gelatinase activity of MMP9, a deregulation in dendritic spine maturation, alterations in mGluR1/5-dependent LTD and altered social behavior. Interestingly in double KO of Fmr1/Mmp9 mouse all these modifications are prevented; suggesting an interrelationship between the activities of both proteins. Furthermore, the level of MMP9 and the activity of FMRP are regulated by glutamate neurotransmission, while the level of FMRP is sensitive to glucocorticoids, an important stress hormone. We evaluated whether the relationship between MMP9 and FMRP observed in KO mice is preserved in an acute restraint stress model. Adult male wild type Sprague-Dawley rats were stressed by restraint during 0.5 or 2.5 hours, and in order to evaluate the posterior effects of stress on hippocampus, another group was sacrificed after 1.5; 6 and 24 hours post stress. During the first 0.5 h of stress we observe an increase in FMRP levels accompanied by a decrease in the levels of MMP9. After 2.5 h of stress FMRP returned to control levels, while MMP9 increased. We did not observe changes in FMRP phosphorylation. At 24 h post stress both protein levels return to normal. We concluded that the relationship between MMP9 and FMRP observed in KO mice is preserved in an acute restraint stress model. These results suggest a cross-regulation mechanism that involves an inverse relationship between both proteins.

Disclosures: F.I. Aguayo: None. **P. Rojas:** None. **A.A. Pacheco:** None. **G.J. García-Rojo:** None. **M. García-Pérez:** None. **M. Muñoz-Llanos:** None. **R. Márquez:** None. **J.L. Fiedler:** None.

Poster

075. Cells and Circuits of Stress

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Topic: E.05. Stress and the Brain

Support: Simons Foundation Award 2448132

NIH Grant 3 UL1 RR024143

NIH Grant 2 R01 NS037466

Title: Finding the missing heritability in GWAS: u-statistics of genetically structured data in subgroup analyses of epilepsy, autism and migraines

Authors: *K. M. WITTKOWSKI[1], E. EISING[3], C. DADURIAN[2], B. BIGGIO[2], G. M. TERWINDT[3], M. D. FERRARI[3], I. HEADACHE GENETICS CONSORTIUM[4], A. M. J. M. MAAGDENBERG[3];
[2]CCTS, Biostatistics, Epidemiology & Res. Design, [1]The Rockefeller Univ., New York, NY; [3]Med. Ctr., Leiden Univ., Leiden, Netherlands; [4]IHGC, Helsinki, Finland

Abstract: Background: A decade after the Human Genome Project, the clinical advances hoped for from genome-wide association studies (GWAS) have not yet been realized. Enlarging sample sizes to tens or hundreds of thousands limits the questions that can be addressed, but does not guard against non-functional SNPs differing between non-randomized populations. Combining a novel computational biostatistics approach with decision strategies fine-tuned to GWAS, we can now identify clusters of related gene in groups of hundreds of subjects only. Methods: U-statistics for genetically structured data account for linkage disequilibrium (LD), varying dominance, and compound heterozygosity within a moving window of SNPs. Replacing the conventional fixed 10E−7.5 level with a study-specific cut-off for genome-wide significance accounts for differences in minor allele frequency, the non-randomized nature of GWAS, and for conducting related tests in overlapping diplotypes. Results: The approach was validated by confirming the known targets of epilepsy drugs in a study comparing 185 childhood cases against publicly available controls. Comparing non-verbal vs verbal cases in the two independent stages of the Autism Genome project, suggested the same ion channels as being involved in disruption of active language development (DALD) as in migraines, in support of the hypothesis that preventing “stranger anxiety” from turning into migraine-like experiences might preventing a behavioral maladaptation leading to lack of language (Wittkowski KM Transl Psychiatry 2014, 4:e354). Conclusions: By reducing sample size requirements for GWAS, the genetic data collected over the last decade can now yield profound insights into the etiology of common diseases and subgroup analyses can rescue ‘failed’ phase 3 trials.

Disclosures: **K.M. Wittkowski:** A. Employment/Salary (full or part-time):; The Rockefeller University. F. Consulting Fees (e.g., advisory boards); Johnson & Johnson. **E. Eising:** None. **C.**

Dadurian: None. **B. Biggio:** None. **G.M. Terwindt:** None. **M.D. Ferrari:** None. **I. Headache Genetics Consortium:** None. **A.M.J.M. Maagdenberg:** None.

Poster

075. Cells and Circuits of Stress

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Program#/Poster#: 75.15/T6

Topic: E.05. Stress and the Brain

Support: Fund for Scientific Research Flanders (FWO) FWO12/PDO/031

Fund for Scientific Research Flanders (FWO) G.0A54.13N

Title: Paracrine signaling via connexin hemichannels contributes to the propagation of ionizing radiation-induced DNA damage in brain microvascular endothelial cells

Authors: *E. DECROCK[1], D. HOORELBEKE[1], M. DE BOCK[1], H. THIERENS[2], B. DESCAMPS[3], C. VANHOVE[3], L. LEYBAERT[1];
[1]Basic Med. Sci. - Physiol. group, [2]Basic Med. Sci. - Med. Physics, [3]Infinity-MEDISIP-iMINDS, Ghent Univ., Gent, Belgium

Abstract: Rardiotherapy is a common treatment for the management of brain tumors. Although modern techniques provide a more precise delivery of radiation, some exposure of normal brain tissue is inevitable. This results in acute vascular damage, primarily characterized by brain microvascular endothelial cell (mEC) dysfunction (activation and cell death) and blood-brain barrier disruption. Importantly, ionizing radiation-induced cellular effects do not remain confined to the cells directly hit by the radiation, but they can be transferred to adjacent non-irradiated cells via intercellular communication pathways, a phenomenon termed the radiation-induced bystander effect (RIBE). Reported RIBE include apoptosis, changes in proliferation, genetic alterations and oncogenic transformation. It is suggested that the propagation of DNA damage, in particular double-strand breaks (DSBs), is a causal agent in these downstream effects. Paracrine factors and direct cell-cell communication via gap junction channels (GJs) are both critical mediators of RIBE. ECs are well-coupled through GJs which are formed by the docking of two hemichannels (HCs). These HCs, i.e. hexameric channels of transmembrane Cx proteins, are commonly closed but may be activated in response to various, mostly pathological, stimuli. They form a diffusive release pathway for paracrine messenger molecules such as ATP into the extracellular environment. We here aimed to explore the role of Cx channels in RIBE in brain mECs. We optimized a set-up in which a well-delineated zone of an adherent rat brain

microvasular endothelial cell line (RBE4) or primary mouse brain capillary ECs was exposed to X-rays (1 and 20 Gy). We investigated kinetics of DSBs (γ-H2AX), in both the irradiated and surrounding non-irradiated bystander zone. The presence of γ-H2AX-positive cells was detected in the bystander zone with a maximum response 3 h post-irradiation, and was reduced in the presence of pharmacological Cx channel blockers or HC-targeting peptides. Reactive oxygen species (ROS) are hypothesized to play a key role in coordinating the RIBE but the mechanism of distant action of these short-lived molecules remains unclear. We here demonstrated that ROS as well as calcium ions (Ca2+) and extracellular ATP are key players in the propagation of the RIBE. We propose that intercellular Ca2+ signaling via Cx HCs (paracrine ATP release pathway) and presumably also via GJs acts as a feed-forward propagation mechanism of ROS production underlying the RIBE. Hence, our results suggest a novel mechanism for radiation-induced damage of brain mECs which involves Cx channels, ATP release and the Ca2+/ROS signaling axis.

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Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.16/T7

Topic: E.05. Stress and the Brain

Support: University of Massachusetts Department of Psychology

Title: Glucocorticoid receptor-regulated transcription of transposable elements

Authors: *R. G. HUNTER[1], B. B. GRIFFITHS[2];
[1]Psychology, Univ. of Massachusetts, Boston, Boston, MA; [2]Developmental and Brian Sci., Univ. of Massachusetts Boston, Boston, MA

Abstract: Stress has been shown to regulate gene expression through epigenetic mechanisms, but little is known about its effects on the 95% of mammalian DNA that does not code for proteins. 50% of this non-coding DNA is comprised of transposable elements (TEs). These elements have been shown to have a number of adaptive roles, including transcriptional regulation and the provision of functional elements in regulatory non-coding RNAs. It has long been proposed that these elements are important in organismal responses to stress. Corticosteroid hormones released in response to physiological and psychological stress are agonists for

glucocorticoid receptors (GRs), which bind to DNA and act as transcription factors. GR binding has been shown to be highly tissue specific. It has been shown in ChIP-sequencing (ChIP-seq) in hippocampal tissue from adrenalectomized, corticosterone challenged rats that only 40% of GR binding is within genes or gene promoters, with the other 60% binding to intergenic regions. Previously, we have shown that some of this intergenic GR binding is to TEs. TE transcription has been shown to be regulated by drugs of abuse, physiological and psychological stress. Further comparison of GR ChIP-seq data with RNAseq data from acute restraint stress and adrenalectomized, corticosterone challenged rats suggests that GR binding results in the altered transcription of TEs in response to acute restraint stress and corticosteroid challenge. While the biological significance of TE transcription resulting from stress-activated GR binding is still largely unknown, it is likely to play a significant role in both the adaptive stress response and potentially in the maladaptive consequences of chronic or uncontrolled stress.

Disclosures: R.G. Hunter: None. **B.B. Griffiths:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.17/T8

Topic: F.02. Animal Cognition and Behavior

Title: Adult vitamin D deficiency does not alter adult neurogenesis but is associated with impaired performance on a hippocampal-dependent task

Authors: *N. GROVES, R. SULLIVAN, P. JOSH, J. MCGRATH, T. BURNE;
Queensland Brain Inst., St Lucia, Australia

Abstract: Vitamin D has been shown to be important for brain development and function, and vitamin D deficiency may lead to adverse brain outcomes, such as poor cognitive function. To address the link between vitamin D deficiency and cognitive impairment, this study assessed the effects of adult vitamin D (AVD) deficiency on adult neurogenesis in the hippocampus; and learning and memory using a hippocampal-dependent task in mice. Adult male BALB/c mice were fed either a vitamin D-deficient diet or a diet containing vitamin D (1,000 IU/kg) for 10 weeks. One cohort of mice were tested for active place avoidance, which is a hippocampal-dependent learning and memory task. This task requires mice to learn to avoid a shock zone in an arena via extramaze cues over 4 days. A second cohort of mice were treated with BrdU for 10 days, and 32 days later, fixed brains were collected to assess the number of newborn surviving mature neurons and the number of proliferating cells in the dentate gyrus using

immunohistology. Hippocampal brain tissue was collected from a final cohort of mice to assess protein changes using iTRAQ proteomics. In the active place avoidance, control mice learnt the location of the shock zone and over 4 days of testing increased their latency to enter the shock zone. However, there was a significant reduction in avoidance latency in AVD-deficient mice. AVD-deficient mice also showed an increase in distance travelled during the 10 min daily task, despite having no baseline locomotor differences. There was no significant difference in the number of newborn surviving mature neurons or proliferating cells within the dentate gyrus between control and AVD-deficient mice. Proteomic analysis indicated changes in the expression of enzymes involved in the production of glutathione, an important antioxidant for the regulation of oxidative stress. Furthermore, there were changes in the expression of proteins relating to excitatory and inhibitory neurotransmitter systems. Consistent with immunohistological results we identified no expression changes in proteins involved in proliferation or neurogenesis. This study showed that AVD deficiency in mice was associated with impaired performance on the active place avoidance task. However, the AVD-deficient mice did not show a reduction in cell proliferation or hippocampal neurogenesis. Further studies are required to investigate the effects of AVD deficiency on oxidative stress and neurotransmission and how they may relate to cognitive impairment.

Disclosures: **N. Groves:** None. **R. Sullivan:** None. **P. Josh:** None. **J. McGrath:** None. **T. Burne:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.18/T9

Topic: E.05. Stress and the Brain

Title: Amygdalar miR-15a regulation of FKBP5 is essential for intact behavioral response to chronic stress

Authors: ***N. VOLK**[1,3], J. PAPE[4], S. BEN-DOR[2], R. ZWANG[1], S. GIL[1], E. B. BINDER[4], A. CHEN[1,3];
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Abstract: MicroRNAs are important regulators of gene expression and were shown to associate with regulation of various psychiatric disorders; however the identity of specific stress-related

microRNAs (miRs), which are recruited to the Ago2 complex at the time of stress induction has yet to be elucidated. In order to identify a genuine connection between specific miRNAs and their target gene`s 3` UTR following chronic stress, we performed immunoprecipitation of the Ago2 complex and analyzed the identity of the entire population of miRNAs and mRNAs following social defeat in the amygdala. Here, we present evidence that exposure of mice to chronic stress leads to a specific increase of miR-15a levels in the Ago2 complex in the amygdala of stressed mice. A concomitant reduction in the levels of miR-15a predicted target, FKBP5 has been observed. Reciprocally, mice expressing reduced levels of miR-15a in the amygdala alongside chronic exposure to stress, exhibit increase anxiety and depression-like behaviors. FKBP5 is a modulator of glucocorticoid receptor (GR) sensitivity, and thus, the link between both miR-15a and FKBP5 might be functionally important in the stress response. Consistent with methylation-dependent regulation of miR-15a, its levels were elevated following *in vitro* treatment with DNA methyltransferase activity inhibitors. Interestingly, methylations of 7 CpGs, located within the promoter region of human miR-15a, were found to correlate with major depression disorders. Taken together our results support an important role for miR-15a in stress adaptation and the pathogenesis of stress-linked psychopathologies.

Disclosures: **N. Volk:** None. **J. Pape:** None. **S. Ben-Dor:** None. **R. Zwang:** None. **S. Gil:** None. **E.B. Binder:** None. **A. Chen:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.19/T10

Topic: E.05. Stress and the Brain

Support: NSF grant IOS 1146853

LA BOR LEQSF(2012-17)-GF-15

Title: Chronic stress modulates the physiological effects of SIRT1 activity in the dentate gyrus of mouse hippocampus

Authors: *D. YU[1], D. HOMIACK[1], L. A. SCHRADER[2,1];
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Abstract: The sirtuins are a family of NAD+ dependent protein deacetylases that are involved in metabolic processes and have been shown to have various functions in neurons, such as DNA

repair and neuronal survival. Previous work in our lab found that chronic variable stress (CVS) significantly increased Sirtuin1 (SIRT1) activity in the dentate gyrus (DG) of rat hippocampus. Direct infusion of sirtinol, a SIRT1 and 2 inhibitor, into the DG prevented CVS-induced memory impairment and depressive-like behavior. The specific function of SIRT1 in the DG, however, is unknown. We hypothesized that SIRT1 activity may affect neuronal properties in the DG of hippocampus slices of control and CVS-treated mice. In this study, we made the surprising finding that pharmacological inhibition of SIRT1 activity increased granule cells excitability in slices from control mice but not CVS-treated mice. This effect in control mice is mediated by voltage- and calcium-dependent large conductance potassium channels (BK channels), as it is blocked by paxilline, the BK channel blocker. In addition, we found that pharmacological inhibition of SIRT1 activity increased spontaneous excitatory postsynaptic current (sEPSC) frequency, but not amplitude, recorded from DG granule cells in slices from control mice. This effect was not observed in CVS-treated mice. The effect on sEPSC frequency in control mice is action potential-dependent. The effect is also blocked by paxilline, suggesting an increase in presynaptic cell excitability mediated by BK channels. On the other hand, pharmacological activation of SIRT1 decreased sEPSC frequency that is TTX-dependent. These results suggest that SIRT1 affects granule cell intrinsic properties and excitatory input through BK channels in control mice but that pathway is impaired in CVS-treated mice. Our previous results showed that SIRT1 activity in the nucleus is increased in CVS, therefore, we propose that CVS-induced long-term SIRT1 over-activity decreases the sensitivity of membrane targets to SIRT1 activity but increases SIRT1 effects in nucleus and transcriptional level. We will investigate this hypothesis in future studies.

Disclosures: **D. Yu:** None. **D. Homiack:** None. **L.A. Schrader:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.20/T11

Topic: E.05. Stress and the Brain

Title: Study of a newly identified molecule that respond to a stress hormone, glucocorticoid

Authors: *K. KOIZUMI[1], K. NAKAO[2], H. NAKAJIMA[3];
[1]Kanazawa Univ., Kanazawa-Shi, Japan; [2]Dept. of Physiol., Saitama Med. Univ., Moroyama, Japan; [3]Ageo Central Gen. Hosp., Ageo, Japan

Abstract: Maternal stress during pregnancy increases secretion of stress hormones such as glucocorticoid (GC). A numbers of studies indicate high level exposure to GC during prenatal period affects neural development and stress response after birth. It causes increasing anxiety and behavioral problems for children (Neurosci Biobehav Rev 53:1-24, 2015). Moreover, recent studies suggest it causes psychiatric disorders such as depression (Front Neurosci. 8: 420, 2015), schizophrenia (Psychopharmacology 214: 89-106, 2011) and autism (Neuroscience and Biobehavioral Reviews 32: 1519-1532, 2008). Although detail molecular mechanisms that link between prenatal stress and these psychiatric disorders are not well understood, GC responding molecules are good candidates to play critical roles in this mechanism. Here, we show a newly identified GC responsive molecule, Fam107B. Injection of GC agonist, DEX, or restraint stress on pregnant mice resulted in reduction of Fam107B mRNA expression in the brain of their embryos. To figure out its function, we study effects of RNAi or over expression of FAM107B in neuron-like PC12 cells and mouse neural cells. We found Fam107B negatively regulate effects of DEX on proliferation, differentiation and migration. Possibly, FAM107B is one of the key molecules that respond to stress or GC and give critical effects on neural development in the embryonic brain.

Disclosures: **K. Koizumi:** None. **K. Nakao:** None. **H. Nakajima:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.21/T12

Topic: E.05. Stress and the Brain

Title: Altered coding- and noncoding RNA expression in the bed nucleus of the stria terminalis of suicide subjects

Authors: *T. M. KLEIN GUNNEWIEK[1], A. JAGER[2], J. C. GLENNON[2], T. KOZICZ[1], A. ASCHRAFI[1];
[1]Dept. of Anat., [2]Dept. of Cognitive Neurosci., Radboudumc, Nijmegen, Netherlands

Abstract: The human stress pathway constitutes a complex system of interconnected brain structures. One of these structures, the bed nucleus of the stria terminalis (BNST), is a limbic region with extensive connectivity with other (sub)cortical brain structures. Previous studies have shown the potential role the BNST plays in processes such as - but not limited to - stress and anxiety, fear learning, and drug seeking behavior. Although changes in expression in various neurotransmitters as well as neuromodulators have been demonstrated in these conditions, a

comprehensive expression profile of the BNST has not yet been made. MicroRNAs (miRNAs) constitute a class of small noncoding units of RNA, each of which able to regulate the expression of up to hundred different genes, and recent studies suggested that they might be involved in fine-tuning gene networks involved in the neuronal stress response. Using Next Generation Sequencing, we analyzed whole transcriptomics of the human BNST, whereby we compared the expression of coding- and noncoding RNA in BNST tissues collected from depressed suicide victims or from individuals who deceased of natural causes (controls). Preliminary results show at least 75 miRNAs with a significant ($P<0.05$) fold change of at least 1.2 changed in depressed suicides as compared to controls. Of these differentially expressed miRNAs we found, 31 were down regulated, whereas 34 were up regulated. Many of these miRNAs identified have been associated with cellular processes involving neuronal stress response, such as mir-34c and mir-124, while others have been linked to cancer (mir-34b, mir-449b), or diabetes (mir-375). In future studies miRNAs with mouse homologs will be validated using qPCR on BNST tissue from stressed- and non-stressed mice. Based on these findings we propose that differentially expressed BNST miRNAs and their associated gene networks may act as critical constituents in the regulation of neuronal stress response.

Disclosures: T.M. Klein Gunnewiek: None. **A. Jager:** None. **J.C. Glennon:** None. **T. Kozicz:** None. **A. Aschrafi:** None.

Poster

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Topic: E.05. Stress and the Brain

Support: NSERC Grant RG-138199

CIHR Grant MOP-119322

Title: Chronic early life stress alters circadian clock gene expression in the adrenal glands and liver of neonatal rats with no change in corticosterone secretion

Authors: *C.-D. WALKER[1], S. KIESSLING[2], L. LIN[3], N. CERMAKIAN[1];
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Abstract: Disrupting maternal behavior in early neonatal life is recognized to have long term consequences on anxiety, social behavior and memory, possibly in part through changes in hippocampal synaptic plasticity and neural activity in the amygdala. The limited bedding (LB) model used in several studies of chronic early life stress postulates that fragmentation of maternal behavior is a critical aspect of the stress experienced by the neonates. However, how fragmentation of maternal behavior translates into changes in neural activity and physiological regulation in the pups is unknown. In the present study, we hypothesized that fragmented maternal behavior might disrupt circadian rhythmicity, i.e. clock gene expression (Bmal1 and Per2) in peripheral tissue oscillators in young pups. Mothers were kept either under normal (NB) or limited (LB) bedding conditions between postnatal day (PND)1-9 and tissues from pups were collected on PND10 after 24hrs of constant darkness at various times over the 24 h cycle (circadian time [CT]2, CT8, CT14, CT22). Maternal behavior was observed daily between PND1-9, 4 times during the light:dark cycle and scored for nursing time, pup grooming and fragmentation. Brain expression of Bmal1 in the central circadian clock (SCN), as detected by *in situ* hybridization, was rhythmic and not modified by maternal condition. However, expression of clock genes in peripheral circadian clocks, both adrenal and liver was significantly altered by LB condition. In the adrenals, the rhythm of Bmal1 and Per2 was maintained but the amplitude was increased in LB vs NB pups, while in the liver, rhythms in Bmal1 and Per2 were completely abolished by the LB condition. Plasma corticosterone secretion showed circadian rhythmicity with no significant differences between NB and LB pups. Fragmentation of maternal behavior was higher in the dark phase compared to the light phase and pup grooming behavior was higher in LB compared to NB mothers in the dark phase. Despite no change in nursing behavior frequency between maternal condition, body weight gain of LB pups was reduced compared to NB pups. Significant alteration in peripheral clock gene oscillators in the liver and adrenal in LB condition suggest that important changes in feeding and/or temperature regulation might mediate the effect of fragmented maternal behavior on adrenal and liver function during neonatal life.

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Poster

075. Cells and Circuits of Stress

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Topic: E.05. Stress and the Brain

Support: NIH Grant R01 MH 104603

EU COST Action CM1103

Tourette Syndrome Association

Kansas Strategic Initiative Grant

Title: 5alpha-reductase mediates stress response circuitry

Authors: *G. L. OSTERHAUS[1], S. C. GODAR[2], L. J. MOSHER[2], C. M. JONES[2], M. BORTOLATO[2];
[1]Univ. of Kansas, Lawrence, KS; [2]Dept of Pharmacol. and Toxicology, Univ. Kansas, Lawrence, KS

Abstract: Stress is a major environmental factor involved in the activation and precipitation of a host of different neuropsychiatric disorders. Ample evidence has shown that stress elicits sexually dimorphic responses, indicating that hormones may be a critical regulatory element of the stress response cascade. For instance, females are more predisposed to depression and anxiety-related behaviors, whereas males are more vulnerable to aggression. However, the underlying molecular mechanisms that govern how steroid hormones shape disease susceptibility and regulate stress responses in a sex dimorphic fashion remain largely elusive. To investigate the role of androgens in stress regulation, we focused on 5alpha-reductase (5AR), the primary enzyme that catalyzes the major rate-limiting step in the synthesis of androgens and several other neurosteroids that mediate stress responses. Notably, 5AR expression is higher in males and is elevated in response to stress. We found that treatment with the 5AR blocker finasteride (FIN) dose-dependently increased depression-like behaviors in both sexes in the forced swim test. Moreover, these changes were accompanied by a significant reduction in the activity of the hypothalamic-pituitary-adrenal axis (HPA), the primary stress response system. In particular, FIN decreased the levels of two central HPA regulatory factors, namely hypothalamic corticotropin-releasing hormone (CRH) and plasma adrenocorticotropin releasing hormone (ACTH). In a separate cohort of animals, we found that FIN markedly reduced saccharin preference, a well-validated paradigm to capture anhedonia, in both male and female rats. Interestingly, FIN-treated animals displayed a progressive reduction in percentage of saccharin consumption throughout the test, suggesting that FIN may block the rewarding properties of saccharin. Taken together, these findings suggest that 5AR may play a critical role in stress response circuitry.

Disclosures: G.L. Osterhaus: None. **S.C. Godar:** None. **L.J. Mosher:** None. **C.M. Jones:** None. **M. Bortolato:** None.

Poster

075. Cells and Circuits of Stress

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Topic: E.05. Stress and the Brain

Support: Marie Curie Actions FP7-IOF-273487

Title: Neurons born during chronic social stress conditions are more vulnerable to a subsequent stress later in life

Authors: *Z. DE MIGUEL[1,2], U. HADITSCH[3], T. D. PALMER[3], A. AZPIROZ[2], R. M. SAPOLSKY[1];
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Abstract: Objective: Numerous studies have examined the effects of stress on the quantity of neurons generated in the adult hippocampus, with most showing a decrease. Few, however, have examined the quality of neurons born during a period of stress. Here we ask whether neurons born despite ongoing stress are like neurons born during control conditions, and whether those neurons are more robust and resilient, or less so to later challenges in life. Methods: CD1 male mice were exposed to dominant cage mates for two weeks with BrdU administration. Ten weeks later, spatial learning was assessed in the Morris water maze. BrdU-ir/NeuN-ir cells, C-fos-ir/NeuN-ir cells, DCX-ir cells and BrdU-ir/NeuN-ir/C-Fos-ir cells were quantified in the dentate gyrus and latencies to find the hidden platform in the spatial learning task were evaluated. In a second experiment newly born cells were labeled using a retroviral vector MLV-CAG-GFP during chronic social stress or control conditions. Eight weeks later animals were exposed to either a second session of chronic social stress or control conditions. Changes in morphology of newly born neurons were examined by determining spine density, dendrite length and number of dendritic branches. Results: Two weeks of social stress increased the number of neurons born during the last five days of stress. Ten weeks after the stress termination, control and stressed animals showed comparable spatial learning acquisition while stressed animals showed a deficit in spatial memory recall. No differences were found in the number of BrdU-ir/C-Fos-ir/NeuN-ir+ neurons. Chronic social stress had no effect on dendritic morphology in neurons born during control conditions; in contrast, such stress decreased spine density and the number of dendrite branches in neurons born during a prior period of stress. Conclusions: Chronic social stress increases the number of neurons generated in the adult dentate gyrus. This effect may be explained by enrichment components inherent to the social interaction that occurs during the social stress model. Despite increased numbers of newly born neurons, the stressed group showed a deficit in spatial memory recall. Additionally, neurons that are born during chronic

social stress conditions may be more vulnerable to recurrent stress experiences later in life. These findings are the first evidence suggesting that newly born neurons during chronic social stress conditions may be more vulnerable to future social stress episodes. However, these findings warrant further examination of whether these effects are intrinsically driven or driven by microenvironmental factors from the niche.

Disclosures: Z. De Miguel: None. **U. Haditsch:** None. **T.D. Palmer:** None. **A. Azpiroz:** None. **R.M. Sapolsky:** None.

Poster

075. Cells and Circuits of Stress

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Topic: E.05. Stress and the Brain

Support: NIH Grant R01 MH104603-01

Kansas Strategic Initiatives Grant

EU COST Action CM1103

Tourette Syndrome Association

Title: 5alpha-reductase 2 plays a key role in reward and stress

Authors: *S. C. GODAR, G. L. OSTERHAUS, L. J. MOSHER, M. BORTOLATO; Pharmacol. and Toxicology, Univ. of Kansas, Lawrence, KS

Abstract: The stress response system elicits multiple adaptive processes that function on threat appraisal and the enactment of coping strategies. However, a host of studies have shown that both rewarding and aversive stimuli elicit the activation of the hypothalamic-pituitary-adrenal axis (HPA), the primary stress response system. These findings suggest that the HPA is a key substrate for physiological and neurobehavioral adaptive functions. Given the large sexual dimorphism in stress regulation, we investigated the role of neurosteroids in mediating the behavioral and neuroendocrinological responses to different stressors. In particular, we examined 5 alpha-reductase (5AR), the primary enzyme that catalyzes the major rate-limiting step in neurosteroidogenesis. In recent studies, we documented that 5AR blockade with finasteride increased depression-related behaviors and anhedonia, but reduced HPA activation. Finasteride, however, blocks both 5AR1 and 5AR2 isoenzymes, which greatly differ in substrate affinity,

activation requirements and neuroanatomical localization. Despite these differences, the specific contribution of each isoenzyme in stress regulation remains largely unknown. To study the role of 5AR2 in stress regulation, we tested the responses of 5AR2 knockout mice to the forced swim and saccharin preference paradigms, two behavioral tasks that capture depression-like and anhedonic behaviors, respectively. We found that 5AR2-deficient mice showed a marked elevation in depression-related behaviors compared to wild-type littermates. Interestingly, finasteride treatment increased depression-like behaviors in wild-type mice, however this compound did not affect 5AR2 mutants in the forced swim task. These findings were also accompanied by a significant reduction in plasma corticosterone levels in 5AR-deficient mice following forced swim stress. Moreover, we found that 5AR2 mutants exhibited a profound reduction in saccharin preference compared to wild-type animals, suggesting a decrease in responses to reward. Collectively, these results suggest that 5AR2 is a key stress mediator and likely contributes to specific depressive- and anhedonic-like effects of finasteride.

Disclosures: **S.C. Godar:** None. **G.L. Osterhaus:** None. **L.J. Mosher:** None. **M. Bortolato:** None.

Poster

075. Cells and Circuits of Stress

Location: Hall A

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Program#/Poster#: 75.26/T17

Topic: E.05. Stress and the Brain

Support: NIMH RO1 MH 048153

RO1 MH 098554

Title: Glucocorticoid receptor and fkbp5 expression and epigenetic variation in teenage and adult suicide

Authors: *H. S. RIZAVI, D. GRAYSON, H. ZHANG, G. N. PANDEY;
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Abstract: An impaired HPA stress response has been linked to depression and increased risk of suicide. The feedback regulation of the HPA axis by glucocorticoids is mediated by mineralocorticoid (MR) and glucocorticoid (GR) receptors. GR may play a more important role in the regulation of the stress response when endogenous levels of glucocorticoids are high because of its high affinity for DEX and a lower affinity for endogenous cortisol. GR-mediated

response is regulated by a number of co-factors which interact with GR and regulate its affinity for cortisol. FKBP5 is a co-chaperone protein that, when bound to GR, decreases its sensitivity to cortisol and therefore affects nuclear translocation. In this preliminary study we wanted to analyze the glucocorticoid receptor expression, specifically variant 1F, and FKBP5 in prefrontal cortex samples obtained from a teenage and adult suicide cohort. In addition we also wanted to examine the methylation status of GR1F and FKBP5 promoter in these samples.In adult suicide we observed a decrease in expression of GR1F and an increase in FKBP5 while total GR did not show any change. In teenage suicide total GR and GR1F were both significantly decreased and FKBP5 showed an increase. GR1F promoter region showed a greater increase in methylation in suicide of the teenage group compared to the adult group and a greater demethylation was seen for FKBP5 in suicide of the teenage group compared to the adult. This preliminary study shows that GR and GR related genes may function differentially in teenagers compared to adults. Our data strengthens the idea that epigenetic variation in GR1F and FKBP5 may play an important role in depression and suicidal behavior. In addition, although the sample number was small, variation in methylation status in the GR1F and FKBP5 promoter regions in teenage compared to adult supports the notion that the neurobiology of teenage suicide may be different from adult.

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Poster

075. Cells and Circuits of Stress

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Topic: E.05. Stress and the Brain

Support: NIH R01MH095972

Title: Optogenetic investigation of the anterior bed nuclei of the stria terminalis (aBST) in the inhibition of the neuroendocrine stress response

Authors: *S. B. JOHNSON, R. M. ANDERSON, M. L. HUFF, S. A. ROMIG-MARTIN, R. M. GLANZ, M. C. MILLER, R. T. LALUMIERE, J. J. RADLEY;
Psychology, Univ. of Iowa, Iowa City, IA

Abstract: A vital component of the emotional stress response is activation of the HPA axis, the neuroendocrine signaling cascade that culminates in the release of glucocorticoids (GCs). During emotional stress, upstream cortical limbic regions modulate HPA output via a combination of neuronal and GC-dependent mechanisms. The fact that these regions do not directly innervate

HPA effector cell groups housed within the paraventricular hypothalamic nucleus (PVH), poses challenges for understanding the circuits and mechanisms accounting for how these influences are conveyed to the stress axis. Previous work utilizing functional neuroanatomical and lesioning approaches has identified a putative site within aBST that may serve as one nodal point for conveying stress-inhibitory information from the limbic cortex to PVH. However, as these studies have been largely inferential and have also provided some conflicting information as to the nature of aBST influences over the stress axis, we employed optogenetic approaches to examine the functional role of the aBST–PVH pathways during acute stress-induced HPA activation. Adult rats received bilateral injections of the nonselective light-activated cation channel, channelrhodopsin (AAV5-hSyn-ChR2[E123A]-eYFP; ChR2 group) or control virus (AAV5-hSyn-eYFP; eYFP group) into the aBST followed by bilateral fiber optic implantation immediately dorsal to the aBST. Four weeks later, animals were subjected to an acute emotional stressor (10 min tail suspension; TS) with concurrent 20Hz 473nm laser stimulation for the duration of TS, and repeated blood samples were collected before and after stress exposure for assay of pituitary-adrenal responses. Following photoexcitation of aBST throughout the 10 min period of TS, ChR2 animals showed blunted HPA responses as compared with eYFP controls, evidenced by a significant decrease in ACTH levels immediately after TS (by 30%, $p < 0.05$) as well as a more rapid decline in post-stress corticosterone levels ($p < 0.05$). In a follow-up experiment involving the same procedures with the exception that fiber optic implants were positioned proximally to PVH, photoexcitation of axon terminals originating from aBST recapitulated the HPA-inhibitory effects of aBST cell body stimulation following stress exposure. Immunohistochemical analysis verified that viral-expressing terminals from aBST neurons were GABAergic, and resided in close apposition with CRF-expressing cells within the parvicellular subdivision of PVH. Together, these initial experiments provide a framework for further investigation of aBST involvement in HPA modulation during acute and chronic stress.

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Poster

075. Cells and Circuits of Stress

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Topic: E.05. Stress and the Brain

Support: FAPESP 2013/24661

Title: Stimulatory action of tobacco-specific carcinogen on the keratinocytes and cancer cells proliferation: neurotransmitters receptor-mediated effects

Authors: *D. G. BERNABE[1], F. VERZA[2], G. MIYAHARA[2], S. OLIVEIRA[3];
[2]Oral Oncology Ctr., [3]Basic Sci., [1]São Paulo State Univ., Araçatuba, Brazil

Abstract: In this study we investigated whether keratinocytes (Hacat) and oral squamous cells carcinoma (OSCC)-derived cell lines (SSC9 and SCC25) are able to produce neurotransmitter norepinephrine (NE) as also the effects of the carcinogen 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) on the NE secretion and OSCC proliferation. Using enzyme-linked immunosorbent assay (Elisa) studies to measure NE concentrations and cell proliferation we demonstrated that supernatant of Hacat, SCC9 and SCC25 showed higher NE levels (6-, 14.9- and 15.1-fold more, respectively) compared to culture media without cells ($p<0.01$). When the cells were stimulated with 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific carcinogen, there were increases in the levels of NE secretion in supernatant by Hacat and SCC25 cells ($p<0.05$), but not by SCC9 cells. NNK (10 μM) induced cell proliferation in the Hacat, SCC9 and SCC25 cell lines and these effects were totally inhibited by blocking ß-adrenergic receptors with propranolol ($p<0.05$). The NNK-induced OSCC cell proliferation was also dependent on nicotinic acetylcholine receptors α4 (nAChR-α4) activation (totally in SCC9 cells and partially in SCC25 cells), but not dependent on nAChR-α7 activation. Inhibition of the ß-adrenergic receptors, nAChR-α4 and nAChR-α7 did not block NNK-induced Hacat proliferation ($p>0.05$). Our findings suggest that NNK-induced alterations in OSCC can be associated with imbalance of norepinephrine secretion and neurotransmitter receptors activations.

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Poster

075. Cells and Circuits of Stress

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 75.29/T20

Topic: E.05. Stress and the Brain

Title: Drug discovery for the treatment of neuropsychiatric disorders using the zebrafish model system

Authors: *J. BURGSTALLER[1], D. BARBER[2], M. SCHÖNBERGER[2], K. SLANCHEV[1], D. TRAUNER[2], H. BAIER[1];

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Abstract: Psychiatric disorders, such as depression, anxiety, schizophrenia and autism, affect a large fraction of the human population. Major depression is characterized by low mood, anhedonia, social withdrawal and other severely debilitating behavioral symptoms. Dysregulation of the hypothalamic-pituitary-adrenal (HPA, "stress") axis is highly correlated with depression and may be a contributing cause to the development of the disease. The identification of a zebrafish (Danio rerio) experimental model with a mutation in the glucocorticoid receptor provides an invaluable tool for research into the pathogenesis of depression, and for the development of potential drug treatments. In this mutant, grs357, the stress axis is chronically elevated (Griffiths et al., 2012), and the homozygous adult animals exhibit behavioral phenotypes reminiscent of an affective disorder (Ziv et al., 2013). Due to their small size, genetic tractability, low cost and quick reproductive cycle, zebrafish have emerged as a new model system for high-throughput *in vivo* screens in search of disease-modifying chemical compounds. We have screened a library of 2,300 biologically active small molecules, at two concentrations, for their acute effects on wildtype and grs357 mutants. Clustering the data by phenotype has revealed a number of novel modifiers of zebrafish larval behavior. Screening of drug X genotype interactions using grs357 is therefore opening up the possibility of identifying novel therapeutic avenues for the treatment of stress-related disorders. In addition, in a more targeted approach, we are exploiting the transparency of zebrafish larvae to explore the utility of photoswitchable, neuroactive compounds to modify behavior. These studies are designed to fulfill the promise of zebrafish as a drug-discovery vehicle for neuropsychiatric disorders.

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Poster

075. Cells and Circuits of Stress

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Topic: E.05. Stress and the Brain

Support: NIH Grant HD075750

Title: Imaging the neural circuitry of life threat in prairie voles

Authors: *J. R. YEE[1,3], W. KENKEL[3], A. PERKEYBILE[3], K. MOORE[1], P. KULKARNI[1], S. W. PORGES[5], C. F. FERRIS[2], C. CARTER[4];
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Abstract: Encounters with fearful and life threatening situations elicit behavioral changes that aid the organism's adaptation and survival. However, these changes can be directionally opposite, ranging from hypermobilization characteristic of fight/flight responses, to immobilization characteristic of freezing responses. Understanding individual differences in the response to life threat may be critical to improving treatments for trauma-related disorders. Prairie voles exhibit several human-like characteristics that may be relevant to understanding the considerable individual differences in biobehavioral responses to fear, including a predilection for strong social attachments and high parasympathetic tone. In preliminary studies, voles exhibited a high degree of individual variation in behavioral and cardiological outcomes when presented with life threat (i.e. exposure to a live ferret). Thus, we sought to investigate whether such differences are represented by differential patterns of neural activation. Using an awake imaging approach, we examined blood oxygen level dependent (BOLD) changes in response to the odor of a live sable ferret. In comparison to an odor with mild positive valence (i.e. almond, 1% benzaldehyde), ferret odor resulted in significant BOLD activation in over 60 regions of interest (out of 117 total), including regions known to be critical to defense behavior such as the central amygdala and periaqueductal gray. Ongoing imaging work will investigate whether individual differences in the behavioral and physiological response to life threat are represented by distinct patterns of BOLD change throughout the brain.

Disclosures: J.R. Yee: None. **W. Kenkel:** None. **A. Perkeybile:** None. **K. Moore:** None. **P. Kulkarni:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ekam Imaging. **S.W. Porges:** None. **C.F. Ferris:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ekam Imaging. **C. Carter:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.01/U2

Topic: E.05. Stress and the Brain

Support: NSERC

Title: Characterization of effects of n3- versus n-6 PUFA rich diets in the juvenile period on emotional responses, corticosterone secretion and neuroendocrine pathways

Authors: *J. RAYMOND[1], H. PLAMONDON[2];
[2]Behavioral Neurosci., [1]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Introduction: N-3 PUFAs such as found in fish oil (FO) play an important role in neuronal growth, synapse function and cognitive abilities. Such effects are observable in both adult and adolescent rats. During the adolescent period, the prefrontal cortex undergoes a prolonged course of maturation lasting well after puberty. This region and the interconnected amygdala, hippocampus and mesolimbic/nigrostriatal systems are then vulnerable to positive and negative environmental conditions, including stress exposure and diet. Objectives: The objective of this study is to determine the impact of FO supplementation on behavioural and neuroendocrine responses in adolescent male rats. Methods: Twenty-nine Wistar rats (n=29) were divided in three groups (n=8-10 per group). Control animals receive soybean oil (C-SO, presenting a n-6/n-3 ratio similar to that found in a balanced diet). Experimental groups receive either fish oil (FO, n-3 PUFA rich condition; containing 300 mg/kg/day DHA) or hydrogenated vegetable fat (HVF; rich in total fatty acids with a high n-6/n-3 ratio, including trans fat). A group with no supplement was also added as a control (Home cage - HC; n=5). 0.3g of supplement per 100g body weight was provided daily to each rats for the entire adolescence period (PD27-PD48) by oral gavage. Blood sample level were collected by a tail nick procedure to measure corticosterone (CORT) levels every week and quantify with an ELISA. Locomotion and anxiety was determined using the Open Field and Elevated-Plus Maze tests. Immunohistochemistry analysis assessed corticotropin releasing hormone, CRH type 1 and glucocorticoid receptor receptor expression in interconnected brain regions including the prefrontal cortex, amygdala, hippocampus and nucleus accumbens. Results: Preliminary data indicate reduced anxiety-like behavior in FO-treated rats as well as a reduce secretion of CRH, glucocorticoids and CORT. Immunohistochemical detection is currently being analysed.

Disclosures: **J. Raymond:** None. **H. Plamondon:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.02/U3

Topic: E.05. Stress and the Brain

Support: NSERC Grant

Title: Social instability stress in adolescence reduces social interactions with novel peers and improves social memory in male rats

Authors: *T. E. HODGES[1], M. L. MARCOLIN[2], J. L. BAUMBACH[3], C. M. MCCORMICK[3]; [1]Psychology, Brock Univ., St Catharines, ON, Canada; [2]Biol., [3]Psychology, Brock Univ., St. Catharines, ON, Canada

Abstract: Adolescent male rats exposed to repeated social instability stress (SS rats; 1 h isolation + return to an unfamiliar cage partner daily for 16 days from postnatal day (P) 30 to P45) and non-stressed controls (CTL rats) were tested at P46 on a social interaction test in an arena with either their cage partner or an unfamiliar rat. There was an effect of stress group ($p < 0.005$), of partner familiarity ($p < 0.001$), and an interaction of the two factors ($p = 0.02$): SS rats interacted less than did controls when with an unfamiliar partner ($p = 0.004$), and did not differ significantly when with their cage partner ($p = .67$). On P47, rats underwent a social memory test. Rats were provided the opportunity to investigate a rat secluded in a wire mesh cage (familiarization phase), and after a retention interval (30, 60, or 90 minutes long), provided with the opportunity to investigate the familiar rat or a novel rat (test phase). SS and CTL rats spent the same amount of time investigating the rat during the familiarization phase ($ps > .31$), but an interaction of stress group and time spent with the familiar or novel rat in the test phase was found ($p = 0.02$). SS rats showed evidence of social memory, spending more time investigating the novel than the familiar rat at every interval ($p < 0.001$), whereas CTL rats spent the same time investigating each rat at every interval ($p = .74$). The heightened social memory may be related to the sensitized corticosterone response SS rats show to unfamiliar conspecifics (Hodges & McCormick, 2015), which may improve social memory (Timmer & Sandi, 2010). The lack of social memory in controls was not due to inability to discriminate between familiar and unfamiliar peers based on the results from the social interaction test. SS rats may require less encoding time than do controls for social memory. Our results add to our previous findings of atypical social behaviour in SS rats in adulthood, and highlight how adolescent experiences shape the social brain.

Disclosures: T.E. Hodges: None. **M.L. Marcolin:** None. **J.L. Baumbach:** None. **C.M. McCormick:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.03/U4

Topic: E.05. Stress and the Brain

Support: NSERC Grant

Title: Social instability stress in adolescence alters the effects of social context on ethanol consumption

Authors: *M. D. MARCOLIN[1], T. E. HODGES[2], J. L. BAUMBACH[2], C. M. MCCORMICK[2]; [2]Psychology Dept., [1]Brock Univ., St Catharines, ON, Canada

Abstract: Social instability stress [SS; 1 h isolation + new cage partners daily from postnatal days (PND) 30 - 45] in adolescence produces long-lasting changes in the rat's social repertoire and in the behavioural responses to psychostimulants (rev. in McCormick et al., 2014). Because ethanol (EtOH) consumption is greater in adolescents than adults and is influenced by social contexts (rev. in Spear, 2014), we investigated whether SS rats differ from control (CTL) rats in EtOH intake and in the effects of EtOH on social behaviour in three experiments. Expt 1 investigated intake in the home cage after stress under conditions of intermittent access to 10% EtOH + 0.1% saccharin (24 h access, 3x a week, from PND46 to PND66) in a two bottle choice paradigm. SS showed reduced EtOH intake and preference than did CTL rats across weeks. Expt 2 investigated intake of EtOH (first exposure, same solution as in Expt 1) in rats on PND47 in a test arena involving different social contexts (alone, with a familiar partner or an unfamiliar partner from the same or different experimental group, with test pairs separated by wire mesh). SS rats spent less time drinking EtOH than did CTL rats, and rats alone drank less than did paired rats, and those paired with an unfamiliar partner drank more than did the other groups. Although the majority of rats' time was spent at the mesh, the group differences in time at the mesh paralleled the EtOH drinking results, thus one behaviour was not performed at the cost of the other. In Expt 3, rats on PND48 were injected with either saline or 10% EtOH (0.5 mg/kg dose) 30 min before placement in a test arena for 15 min with a non-injected unfamiliar CTL. EtOH increased time spent in social interaction compared with saline in CTL rats, and EtOH decreased time spent in social interactions compared with saline in SS rats. The differences in drinking may involve the altered social repertoire of SS rats or may involve altered EtOH sensitivity (e.g., hedonic value, EtOH metabolism) in SS rats.

Disclosures: M.D. Marcolin: None. **T.E. Hodges:** None. **J.L. Baumbach:** None. **C.M. McCormick:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.04/U5

Topic: E.05. Stress and the Brain

Support: NIH 5-T32-NS007413-17

NIH R01MH093981-03

Title: The impact of repeated social stress on the juvenile prefrontal cortex: neuronal function and synaptic communication

Authors: *K. R. URBAN[1], J. LI[2], R. J. VALENTINO[1];
[1]Gen. Anesthesia, Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Bioengineering, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Chronic or repeated stress can lead to the development of psychiatric illnesses including depression, post-traumatic stress disorder, schizophrenia, and anxiety.Cognitive function is impaired in these disorders and this is thought to arise in part from stress-induced alterations in cells and circuitry of the prefrontal cortex. Previous studies have shown that an acute stressor can enhance glutamate transmission and cognition, whereas chronic stress reduces glutamate transmission and impairs cognition. However, the bulk of existing research has been performed on adult, male rats, and has not attempted to elucidate distinct age-dependent or sex-dependent mechanisms of stress effects on cognition. Little is known about how age at the time of stress may impact the outcome. Additionally, no studies have examined the effects of social stress, a relevant stressor for humans. In this study, rats were exposed to 5 consecutive days of resident-intruder stress (30 min/day) or control manipulation during early adolescence (32-38 days old, EA), mid-adolescence (42-48 days old, MA), or adulthood (68-72 days old, adult). One day after the final defeat, cellular characteristics of layer 5 pyramidal neurons in the prefrontal cortex were examined using whole-cell patch clamp in 300 μM brain slices. It was found that repeated social stress increased input resistance and half-width of action potentials selectively in MA rats. Although the threshold to fire action potentials was lower in neurons from MA stressed rats, the neurons fired for a much briefer period than those from control rats. In addition, the amplitude, but not frequency, of spontaneous excitatory post-synaptic currents (sEPSCs) was lowered in EA and MA stressed rats, but not in adults. These results suggest that repeated social stress decreases the overall responsiveness of pyramidal neurons potentially by altering the function of both sodium and potassium channels. In addition, the reduced amplitude of sEPSCs suggests reduced expression of postsynaptic glutamate receptors, which could result in impaired plasticity and cognitive function. These data provide the first age-dependent examination of repeated social stress effects on the cellular function of the prefrontal cortex, and suggest that late adolescence/puberty is a period of particular vulnerability to the effects of social stress. They also suggest cellular mechanisms for the cognitive impairments seen following chronic stress.
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Disclosures: **K.R. Urban:** None. **J. Li:** None. **R.J. Valentino:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.05/U6

Topic: E.05. Stress and the Brain

Support: NIH Grant R21MH091445

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Klarman Family Foundation Grant Program in Eating Disorders Research

P30 EY13079

Fulbright Graduate Study Grant

Title: The effects of food restriction and exercise on anxiety-related behaviors and cognitive functions in male and female adolescent mice

Authors: *Y.-W. CHEN, C. AOKI;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Adolescence is the least studied stage of brain development and is especially so for females, even though many mental illnesses emerge for the first time among this group. Anxiety is 60% more prevalent in females and is a core feature of many mental illnesses, including anorexia nervosa (AN). The incidence of AN is most common in adolescent females, suggesting an effect of hormones, age, and sex in vulnerability to the disease. There is yet no accepted pharmacological treatment for AN, reflecting our lack of understanding of the underlying neurobiology of AN or of anxiety. Anxiety is a double-edged sword: it can promote a crippling focus upon negative life-events, leading to impairment in cognitive functions, while great reductions of stress-induced anxiety may impair learning and memory. Activity-based anorexia (ABA) is an animal model for identifying the biological basis of excessive exercise and starvation, 2 hallmarks of AN. It was recently shown that 24 hours of food restriction, with or without exercise, reduced anxiety significantly in female adolescent mice. However, the effects of exercise and food-restriction separately or jointly as ABA on anxiety-related behaviors in male adolescents have never been tested. Moreover, how these anxiety-related level changes in adolescence would affect the cognitive functions later in life remains largely unknown. Here,

four experimental groups of C57BL/6 male and female pubertal mice were evaluated: ABA (housed with a running wheel from P36-44 and food restriction from P41-44) and three control groups: CON (control mice with no running wheel experience or food restriction), EX (housed with a wheel from P36-44), and FR (food restricted from P41-44). Three-way ANOVA was run, using food restriction, exercise and sex as the factors. We assessed anxiety-related levels of these animals before ABA (P39, open field), during ABA (P42, marble burying), and after recovery from ABA (P51, elevated plus maze). Spatial and object recognition memory and social preference and recognition of the animals were tested after recovery from ABA (P53-P57). 24 hours of food restriction (at P42) reduced anxiety significantly in both male and female adolescent mice, while exercise had no effect. One week after recovery from ABA, males of the FR, EX, and ABA groups showed increased anxiety compared to CON, but females did not. However, anxiety impacted negatively on spatial memory performance in females, but no such correlation was found among males. This study highlights sex differences in response to food restriction and/or exercise during adolescence and provides the foundation for the next study that will examine the effect of val66met SNP of BDNF.

Disclosures: Y. Chen: None. **C. Aoki:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.06/U7

Topic: F.02. Animal Cognition and Behavior

Support: Behavior & Brain Research Foundation

NIH HD055177

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NIH NS052819

NIH 1R01HD076914

NIH R00 MH097822-03

Title: Leveraging dynamic changes in neural circuitry during adolescence to persistently attenuate fear memories

Authors: *S. S. PATTWELL[1], C. LISTON[2], D. JING[2], I. NINAN[3], R. YANG[2], B. CASEY[2], K. DEISSEROTH[4], F. S. LEE[2];
[1]Human Biol., Fred Hutchinson Cancer Res. Ctr., Seattle, WA; [2]Weill Cornell Med. Col., New York, NY; [3]NYU Sch. of Med., New York, NY; [4]Howard Hughes Med. Institute, Stanford Univ., Stanford, CA

Abstract: Fear can be highly adaptive in promoting survival, yet it can also be detrimental when it persists long after a threat has passed. Malleability of the fear response may be most advantageous during adolescence when animals are prone to explore novel, potentially threatening environments. Two opposing adolescent fear-related behaviors_diminished extinction of cued fear and suppressed expression of contextual fear_may serve this purpose, but the neural basis underlying these changes is unknown. Using microprisms to image prefrontal cortical spine maturation longitudinally, we delineate dynamic BLA-hippocampal-mPFC circuit reorganization associated with these behavioral shifts. Exploiting this sensitive period of neural development, we devised a behavioral intervention to attenuate adolescent fear memories persistently into adulthood. These findings define a strategy for leveraging dynamic neural changes during adolescence to extinguish pathological fears implicated in anxiety and stress related disorders.

Disclosures: **S.S. Pattwell:** None. **C. Liston:** None. **D. Jing:** None. **I. Ninan:** None. **R. Yang:** None. **B. Casey:** None. **K. Deisseroth:** None. **F.S. Lee:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.07/U8

Topic: E.05. Stress and the Brain

Support: Israel Science Foundation (484/10)

Title: The anxiogenic effects of pre-reproductive stress (PRS) in female rats on their female offspring are partially reversed by maternal post-stress treatment with fluoxetine and NBI 27914

Authors: *H. ZAIDAN, I. GAISLER-SALOMON;
Psychology, Univ. of Haifa, Haifa, Israel

Abstract: Studies in humans and animals indicate that stress can affect behavior and hypothalamic-pituitary-adrenal (HPA) axis function in subsequent generations. We have

previously shown that prereproductive stress (PRS) to female rats in late adolescence impacts her offspring's anxiogenic behavior in a sex-dependent manner. We further showed that stress increases both corticosterone (CORT) levels and corticotropin releasing factor 1 (CRF1) expression in the frontal cortex and oocytes of the exposed females, as well as in their offspring's brain at birth. Here, we aimed to test whether maternal treatment with the CRF1 antagonist NBI 27914 (NBI, 5 mg/ml; 5 days) or the antidepressant drug fluoxetine (FLX, 5 mg/kg, 7 days) would reverse the behavioral alterations induced by adolescent PRS on dams and their offspring. Adolescent female rats (F0) were exposed to a 7-day stress procedure. Twenty four hours later, PRS and control rats were put on a subchronic drug treatment regimen with FLX, NBI or vehicle. Female rats (F0) and their offspring (F1) were weighed regularly, and behavior in dams and offspring was assessed on P60. In F0, PRS increased weight gain and decreased locomotion in the open field (OF), which were unaffected by drugs. In offspring, maternal PRS and drug treatment led to sex-dependent effects. Female offspring of PRS (O-PRS) displayed an increase in the latency to enter the open arms in the elevated plus maze (EPM) as well as abnormal acquisition of fear conditioning (FC), which were reversed by both NBI and FLX treatment. FLX also normalized the maternal PRS-induced increase in freezing in the OF. In males, maternal PRS enhanced open arm frequency in the EPM, and led to less freezing in the acquisition and context retrieval phases of FC. In the OF, O-PRS males displayed enhanced freezing and decreased center time duration. These behaviors were not affected by maternal drug treatment. Interestingly, maternal drug treatment on its own affected offspring weight and behavior in the FC test as well as in assays of novel object and social exploration. In sum, our findings indicate that PRS to adolescent female rats affects offspring behavior, and that administration of FLX and/or NBI immediate after stress reverses some of these effects in female offspring alone. O-PRS males display behavior that may be interpreted as "enhanced risk-taking", and is unaffected by drug treatment. Drug treatment on its own also impacts offspring behavior. These findings indicate that some of the transgenerational effects of pre-gestational trauma may be prevented by post-stress drug treatment, and that different mechanisms may mediate the inheritance of stress to male and female offspring.

Disclosures: H. Zaidan: None. **I. Gaisler-Salomon:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.08/U9

Topic: E.05. Stress and the Brain

Support: NIH Grant R15 MH100689 (JJQ)

Harlan Scholars Program

Title: Impact of juvenile stress on adult fear learning and dendritic morphology in the basolateral amygdala

Authors: *R. A. SKIPPER[1], C. L. WELLMAN[1], M. R. HERBST[2], J. J. QUINN[2];
[1]Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [2]Dept. of Psychology, Miami Univ., Oxford, OH

Abstract: In humans, stress can lead to a host of debilitating outcomes, such as specific phobias, anxiety disorders, and post-traumatic stress disorder. These maladaptive effects have been modeled in rodents, with acute stress resulting in enhanced contextual fear conditioning and dendritic retraction in the amygdala. However, little research has been done to understand the effects of early life stress on emotional learning in adults. We hypothesized that juvenile stress would result in long-term behavioral and morphological effects: enhanced fear conditioning and dendritic alterations in the basolateral amygdala (BLA) in adulthood. Male and female Long-Evans rats were either stressed or unstressed, with equal numbers of males and females in each group. On postnatal day 17 (PND17), stressed rats received 15 mild footshocks in Context A; unstressed controls were exposed to the same context without footshock. All then matured without manipulation until adulthood. On PND90, rats underwent cued fear conditioning (one tone-shock pairing) or no conditioning (one presentation of tone alone) in Context B. On subsequent days, rats underwent tests of contextual fear conditioning (Context B), tone fear conditioning (novel Context C), and memory for early life stress (Context A). In all tests, fear memory was measured as freezing during context or tone exposure. Upon completion of behavioral testing, rats were euthanized and brains were stained using Golgi histology. Dendritic morphology of BLA pyramidal neurons was assessed. Stressed rats showed enhanced contextual fear conditioning compared to unstressed rats. This was accompanied by increased dendritic length and complexity in BLA pyramidal neurons. Overall, cued fear conditioning did not differ between stressed and unstressed rats. However, this could be due to sex differences in fear responding. Preliminary data suggest that males showed more robust cued fear conditioning compared to females, and this difference paralleled differential effects of early stress on dendritic morphology in BLA. These results suggest that the dendritic remodeling implicated in adult stress-induced behavioral changes may also be involved in the maladaptive effects of early life stress. This is relevant to the effects of early life stress and improper fear regulation among humans, including individuals with early-developing post-traumatic stress disorder or those recovering from childhood trauma.

Disclosures: R.A. Skipper: None. **C.L. Wellman:** None. **M.R. Herbst:** None. **J.J. Quinn:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.09/U10

Topic: E.05. Stress and the Brain

Support: CIHR

IMHR Graduate Student Bursary

Title: Access to palatable food alters gastrin-releasing peptide and dopamine signalling in the medial prefrontal cortex of adult rats exposed to juvenile stress

Authors: *E. ALI[1,2], J. C. MACKAY[3], M.-C. AUDET[2], J. S. JAMES[2], C. CAYER[2], P. KENT[2,3], Z. MERALI[2,3];
[1]Carleton Univ., Nepean, ON, Canada; [2]Inst. of Mental Hlth. Res., Ottawa, ON, Canada; [3]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Stress-exposure during juvenility increases the susceptibility for anxiety and depressive characteristics in adulthood and is also thought to contribute to the growing prevalence of obesity in school-aged children. Stress can increase food intake and a preference for highly caloric palatable food (PF), contributing to weight gain and a risk of developing obesity while PF has stress-buffering characteristics shown in humans and rodents. A feeding peptide called gastrin-releasing peptide (GRP) is involved in stress response and also reduces feeding. GRP receptors (GRPR) are expressed in the medial prefrontal cortex (mPFC), an area implicated in stress and motivated behaviour. Dopamine receptors 1 and 2 (DRD1 and DRD2) are expressed in the mPFC and are involved in reward, motivation and stress. The aim of the study is to reveal the long-term effect of juvenile stress and access to PF on behavioural and metabolic measures and GRP, GRPR, DRD1 and DRD2 mRNA expression in the mPFC. Male Wistar rats that underwent episodic stressor exposure on postnatal days (PD) 27-29 displayed social anxiety that was reversed by consumption of PF. In contrast to the stress buffering effect, PF in combination with previous stressor exposure led to increased weight gain and accumulation of body fat at PD 60. Qualitative polymerase chain reaction revealed an increase of GRP mRNA expression and no change in GRPR expression in juvenile stressed rats with access to PF. The same group also revealed an increase in DRD2 mRNA expression, whereas DRD1 expression was decreased in juvenile stressed rats irrespective of diet. In conclusion, PF mitigates the effects of juvenile stress in adulthood at the expense of increased adiposity in part due to a dysregulation of GRP and dopamine signaling.

Disclosures: **E. Ali:** None. **J.C. MacKay:** None. **M. Audet:** None. **J.S. James:** None. **C. Cayer:** None. **P. Kent:** None. **Z. Merali:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.10/U11

Topic: E.05. Stress and the Brain

Support: Project PRIN 20107MSMA4

Title: Changes in the regulation and function of the HPA axis induced by juvenile social isolation in rats

Authors: ***M. SERRA**[1], G. BOERO[3], F. BIGGIO[2], M. PISU[4];
[1]Univ. of Cagliari, Monserrato (Cagliari), Italy; [2]Univ. of Cagliari, Cagliari, Italy; [3]Univ. of cagliari, Cagliari, Italy; [4]Inst. of Neurosci. CNR, Cagliari, Italy

Abstract: Rearing of rodents from weaning to adulthood in isolation induces various behavioral and neurochemical alterations in comparison with group-housed controls, suggesting that socially isolated rodents represent an animal model of neuropsychiatric disorder. Namely, social isolation results in a decrease in the brain and plasma concentrations of neuroactive steroids that act as positive modulators at GABAA receptors and is accompanied by an enhanced response to the acute administration of ethanol as well as by enhanced neurosteroidogenesis in response to acute stressful stimuli. It also increases the sensitivity of the pituitary to corticotropin-releasing factor (CRF) and impairs negative feedback regulation of the HPA axis. The aim of this research was to study mechanisms that might contribute to the different HPA reactivity to acute stress. We evaluated plasma corticosterone, hypothalamic level of CRF, and the expression of hippocampal and hypothalamic glucocorticoid receptors (GR) at different time point after acute heterotypic stress (foot-shock, 5 min). In group-housed animals plasma corticosterone levels were significantly increased after 5 min, reached at peak at 15 min and returned to baseline 60 min after stress. At variance, plasma corticosterone levels of socially isolated rats were increased at 5 min, after stress, were maximum at 60 min and remained significantly high 7 hours later. In the hypothalamus of group-housed animals no changes in the levels of CRF were detected in any time point measured, while in socially isolated rats a significant increase of hypothalamic CRF levels was found at 15 min, 30 and 60 min after the end of the stress. These data demonstrate that social isolation results in hypothalamic hyperactivity under acute heterotypic stress and suggest that socially isolated rats are much less sensitive to glucocorticoid negative feedback than are

group-housed animals. Social isolation increased basal levels of both hippocampal and hypothalamic membrane GR. Moreover, in group-housed rats GR expression progressively increased with the time after foot-shock exposure, becoming statistically significant 90 min after stress, while socially isolated rats did not show any change in hippocampal and hypothalamic GR expression at any time point examined. These results suggest that dysregulation of GR expression induced by socially isolation stress may cause modifications in the activity of HPA axis and in the negative feedback resulting in prolonged responses to acute stress.

Disclosures: **M. Serra:** None. **G. Boero:** None. **F. Biggio:** None. **M. Pisu:** None.

Poster

076. Stress in Juveniles and Adolescents

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 76.11/U12

Topic: E.05. Stress and the Brain

Support: DIB, Universidad Nacional de Colombia

Title: Overcrowding causes variations in hair corticosterone concentration in juvenile male Wistar rats

Authors: ***M. J. ROJAS**[1], D. GONZÁLEZ-UARQUIN[2], L. F. CARDENAS[3], J. S. MEYER[4]; [1]Salud Animal, [2]Grupo de Neurociencias, Univ. Nacional Colombia, Bogota, Colombia; [3]Univ. de los Andes, Bogota, Colombia; [4]Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Chronic stress due to reduction of the minimum living space for an individual during youth triggers several metabolic and behavioral pathologies in adulthood. The aim of this study was to determine whether a chronic stress condition (overcrowding) induces changes on plasma and hair corticosterone concentration from young Wistar rats. The experimental subjects were divided in two groups (control CG, and overcrowded OG); OG subjects were exposed to an overcrowding period from post-natal day (PND) 38 to PND 65. According to the enzyme immunoassay kit, OG showed a higher corticosterone plasma concentration, and also a higher hair corticosterone concentration compared to CG. These results contribute to demonstrate that the concentration of corticosterone in hair from rats is a suitable, and not invasive method for monitoring chronic stress.

Disclosures: **M.J. Rojas:** None. **D. González-Uarquin:** None. **L.F. Cardenas:** None. **J.S. Meyer:** None.

Poster

077. Thermoregulation and Energy Metabolism

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.01/U13

Topic: E.04. Autonomic Regulation

Support: NIH Grant R01 DA-026867

IUPUI RSFG

iM2CS-GEIRE

Purdue Research Foundation (PRF) Research Award

Title: Circadian variability of body temperature responses to Methamphetamine (Meth)

Authors: *A. BEHROUZVAZIRI[1], Y. YOO[1], E. MOROZOVA[2], M. ZARETSKAIA[3], D. RUSYNIAK[3], D. ZARETSKY[3], Y. MOLKOV[1];
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Abstract: Vital parameters of living organisms exhibit circadian rhythmicity. Despite rats are nocturnal animals, most of drugs of abuse studies in rodents are performed during the day. There is virtually no data on the circadian variability of amphetamine responses. Experiments were performed on male Sprague-Dawley rats implanted with telemetric probes reporting body temperature. Rats received i.p. injections of Meth (1 or 5 mg/kg) or saline at 10-11am or at 10-11pm. Each rat received only one injection of Meth to avoid the effects of repeated administration. The responses were recorded for at least 5 h. The baseline body temperature at night was 0.8ºC higher than during the day. The body temperature increased slightly after injections of saline in both day and night and returned to baseline within 1 h. Both during the day and at night 1 mg/kg of Meth induced monophasic hyperthermia, however, compared to daytime the maximal increase in temperature was half at night. Injection of 5 mg/kg of Meth in the daytime caused a delayed hyperthermic response, preceded by a slight increase of the body temperature immediately after injection. In contrast, the same dose at night produced variable responses with a bias towards a decrease of body temperature. Recently, using mathematical modeling, we showed that the complex dose-dependence of day-time temperature responses to Meth results from a delicate balance between inhibitory and excitatory drives which have different sensitivity to the drug (Molkov et al. Am. J. Physiol. 2014). In this study, we extended

our model by introducing circadian inputs to interpret the night time data above. We have found that during the night the baseline activity of the excitatory response component is greater than during the day. Besides, during both day and night, the equilibrium body temperature after either dose of Meth was noticeably lower than the body temperature observed before injection. The suppression of the hyperthermia/conversion to hypothermia in response to Meth at night is explained by a combination of two factors. First, the excitatory drive appears partially saturated, and, thus, is activated by Meth to a lesser extent. This evokes smaller increase of body temperature in response to low doses of Meth, or the excitatory components becomes overpowered by the inhibitory drive in response to the higher dose. Second, the reduction of the equilibrium temperature leads to gradual cooling which counteracts the hyperthermia/facilitates hypothermia. We speculate that the reduction of the equilibrium body temperature by Meth reflects a suppression of spontaneous locomotor activity underlying circadian variations of body temperature.

Disclosures: **A. Behrouzvaziri:** None. **Y. Yoo:** None. **E. Morozova:** None. **M. Zaretskaia:** None. **D. Rusyniak:** None. **D. Zaretsky:** None. **Y. Molkov:** None.

Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.02/U14

Topic: E.04. Autonomic Regulation

Title: N-methylserotonin from Japanese pepper (Zanthoxylum piperitum), soy isoflavones, black cohosh extract, and combinations thereof regulate skin temperature in a female rat model of menopause-related hot flash

Authors: ***M. J. WEISER**[1], V. GRIMSHAW[1], K. WYNALDA[1], M. H. MOHAJERI[2], C. M. BUTT[1];
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Abstract: Oral administration of soy isoflavones or black cohosh extract for the relief of menopause symptoms have some clinical support, but the data are mixed. Soy isoflavones such as genistein can regulate menopause-related hot flashes, but the mechanisms for this activity are not completely understood. Similarly, black cohosh contains N-methylserotonin (NMS) as a minor component, and NMS has selective agonist activity at the 5-HT_7 serotonin receptor subtype that is involved in thermoregulation. Soy isoflavones and black cohosh are commonly used as homeopathic approaches to menopause symptoms, but it is unknown whether they work

together in additive or synergistic fashions. We have performed three studies that sought to determine the effects of dietary NMS, or its combination with soy isoflavones and black cohosh, on induced hot flash and measures of mood in female rats. All three studies used ovariectomized (OVX) female rats that were fed diets containing different levels of NMS, soy isoflavone concentrate, or black cohosh extract. OVX animals given estradiol implants served as positive controls. Locomotor activity (open field), anxiety-like behaviors (elevated plus maze; EPM), and depression-like behaviors (forced swim test) were assessed first, and then skin temperature was monitored during experimental hot flashes that were induced with intravenous calcitonin gene-related peptide. None of the dietary manipulations affected OVX-induced weight gain, uterine growth, or mood-related behaviors, but estradiol implants increased the time spent in the center of the open field and on the open arm of the EPM. Furthermore, synthetic NMS, NMS contained in Japanese pepper (*Zanthoxylum piperitum*; a 25-fold more efficient source of NMS than black cohosh), black cohosh extract, soy isoflavones and estradiol implants all individually blunted the hot flash response. Dietary combinations of Japanese pepper with isoflavones, black cohosh, or isoflavones + black cohosh also reduced the hot flash response, but the pepper alone and the pepper + isoflavones combination were the most effective. These three separate studies provided replication and confirming results that a natural dietary source of NMS was equivalent to synthetic NMS in reducing the hot flash response, that soy isoflavones and black cohosh can have similar, but less robust, effects on hot flash, and that some benefits may be conferred by combinations of these natural products. These *in vivo* findings also support NMS as a component of black cohosh and Japanese pepper that may reduce menopause-related hot flashes while avoiding the potential side-effects of phytoestrogens or hormone replacement therapy.

Disclosures: **M.J. Weiser:** A. Employment/Salary (full or part-time):; DSM. **V. Grimshaw:** A. Employment/Salary (full or part-time):; DSM. **K. Wynalda:** A. Employment/Salary (full or part-time):; DSM. **M.H. Mohajeri:** A. Employment/Salary (full or part-time):; DSM. **C.M. Butt:** A. Employment/Salary (full or part-time):; DSM.

Poster

077. Thermoregulation and Energy Metabolism

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Program#/Poster#: 77.03/U15

Topic: E.04. Autonomic Regulation

Support: NIH R01DA026867

C06 RR015481-010

IM2CS-GEIRE

IUPUI RSFG

PRF Research Award

Title: Amphetamine enhances endurance by increasing heat dissipation

Authors: *Y. YOO[1], E. MOROZOVA[2], A. BEHROUZVAZIRI[1], M. ZARETSKAIA[3], M. B. BROWN[4], D. RUSYNIAK[3], D. ZARETSKY[3], Y. MOLKOV[1];
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Abstract: Amphetamine is widely used among athletes to enhance performance despite how poorly understood are its ergogenic mechanisms. This study sought to determine if amphetamine may impart performance benefit by impacting thermoregulation during exercise. Two groups of six rats were tested during the experiment, one group (Amph) was injected with amphetamine (2 mg/kg i.p.) and the other group (Saline) – with a saline. Following dosing, rats performed an incremental treadmill test to exhaustion at room temperature (24^{o}C with workload (speed and incline) increases in three minute stages. Continuous recordings of core body temperatures (T_c) via implantable telemetry, and oxygen consumption (VO_2) via analysis of expired gases were performed. Time to exhaustion (mean±SE minutes) for Amph (17.3±0.6) was longer than for Saline (14.8±0.8). T_c in Amph was lower than in Saline at every treadmill workload, however, with the longer running time in Amph, final T_c was not significantly different between groups. Interestingly, for Saline rats T_c increased at a constant rate, while for Amph rats the rate of increase in T_c was increased with workload. Total heat produced, which was calculated from VO_2, was not significantly different between Saline and Amph at every treadmill workload. To further examine these findings, we extended a previously constructed mathematical model with two heat producing compartments – core body and skeletal muscles. Using Bayesian approach we quantified separately the heat generation in the core and muscles, and the heat dissipation, and then compared these parameters between Saline and Amph. The model satisfactorily reproduced and explained the difference of body temperature dynamics between groups. Modeling revealed that amphetamine increased the heat dissipation from the core body. At exhaustion, the mean T_c in both groups were approximately 40 °C. However, muscle temperature in Amph, which was predicted by the model, was higher than in the Saline group, vs. . We concluded that amphetamine (2 mg/kg) increases heat dissipation during exercise thereby slowing down rise in core body temperature. Given the limitation imposed by high core body temperature on time to exhaustion, amphetamine may increase endurance performance by delaying hyperthermia. However, running under the influence of amphetamine may increase risk for skeletal muscle-specific heat injury.

Disclosures: **Y. Yoo:** None. **E. Morozova:** None. **A. Behrouzvaziri:** None. **M. Zaretskaia:** None. **M.B. Brown:** None. **D. Rusyniak:** None. **D. Zaretsky:** None. **Y. Molkov:** None.

Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.04/U16

Topic: E.04. Autonomic Regulation

Support: R01 AG047887

Title: Estradiol modulates temperature regulation in the female mouse

Authors: E. M. BLACKMORE[1], S. J. KRAJEWSKI-HALL[1], *N. E. RANCE[1,2];
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Abstract: Hot flushes are due to estrogen withdrawal and are characterized by the episodic activation of heat dissipation effectors. Despite the vast numbers of individuals affected, there is little understanding of how estrogen modulates thermoregulation in any species. There is also limited information on how estradiol regulates body temperature in the mouse, a valuable research model because of the availability of transgenic lines. In this study, we report a method to record tail skin temperature (T_{SKIN}) in freely-moving mice. Mice were ovariectomized (OVX) and implanted s.c. with silastic capsules containing vehicle or 17β-estradiol (E_2) producing low levels of serum estradiol. Core temperature (T_{CORE}) and activity were measured with an i.p. telemetry probe and T_{SKIN} was measured with a data-logger protected by a Delrin housing, similar to a method we developed for the rat (Williams et al. Endocrinology, 2010). In the first experiment, T_{CORE}, T_{SKIN} and activity were recorded continuously over a five day period in mice housed in their home cages (12 hour light/dark) with ambient temperature ($T_{AMBIENT}$) at approximately 24.0°C. Days 3-5 after capsule implantation, E_2 significantly lowered the T_{CORE} during the light phase (OVX 36.8 ± 0.11°C, n = 10 vs OVX + E_2, 36.4 ± 0.08°C, n = 9, mean ± SEM) but not the dark phase (OVX, 37.9 ± 0.11°C, n = 10 vs OVX + E_2, 37.7 ± 0.06°C, n = 9). Continuous recording of T_{SKIN} revealed wide fluctuations in vasomotion and diurnal rhythms. There was no effect of E_2 on T_{SKIN} or activity during the circadian rhythm recordings. In a second experiment, T_{CORE}, T_{SKIN} and activity were recorded (during the light phase) in mice exposed to a wide range of $T_{AMBIENT}$ (20 - 36°C) in an environmental chamber. OVX + E_2 mice maintained T_{CORE} at a lower level at nearly all $T_{AMBIENT}$, compared to vehicle controls. In addition, the overall T_{SKIN} of OVX + E_2 mice was lower than that of OVX mice, with significant

differences at $T_{AMBIENT}$ of 21, 23, 25 and 26°C. There was no significant difference in activity between the two groups. Overall, our studies show that E_2 treatment of OVX mice consistently lowers T_{CORE} during the light phase. These findings are different than those previously observed in the rat, where T_{CORE} is only lowered by E_2 during heat stress. Our methods to record T_{SKIN} in freely-moving mice will enable further research on the effects of E_2 on thermoregulation in transgenic mouse models.

Disclosures: E.M. Blackmore: None. **S.J. Krajewski-Hall:** None. **N.E. Rance:** None.

Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.05/U17

Topic: E.04. Autonomic Regulation

Title: Heat shock factor 1-deficiency affects systemic control of body temperature

Authors: *A. STAHR[1], M. INGENWERTH[1], E. NOICHL[2], H.-W. KORF[2], H. REINKE[3], C. VON GALL[1];
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Abstract: The hypothalamus controls the autonomous nervous system as well as the endocrine system and regulates metabolic processes, circadian rhythms, behaviour and body temperature. The heat shock (HS) pathway, mediated by the transcription factor HSF1, is an essential defensive mechanism against acute heat exposure and is involved in many cell-signalling pathways to maintain cellular homeostasis. However, little is known about the role of HSF1 in the systemic control of energy homeostasis. Therefore, we analysed core body temperature and spontaneous locomotor activity in HSF1- deficient ($HSF1^{-/-}$) and wildtype littermates ($HSF1^{+/+}$). $HSF1^{-/-}$ mice showed a decrease in locomotor activity and an increase in core body temperature, suggesting a role of HSF1 in the systemic control of energy expenditure. HSF1 is expressed in various hypothalamic brain regions which are involved in regulation of energy metabolism and co-localized with tyrosine hydroxylase, the rate limiting enzyme in dopamine synthesis. HSF1-deficient mice show decreased prolactin levels and increased expression of uncoupled protein 1 in brown adipose tissue. In conclusion, we showed for the first time that HSF1 plays a role in systemic regulation of energy expenditure, hypophyseal prolactin release and peripheral UCP1

expression. Thus, HSF1 might be involved in hypothalamic temperature-mediated feedback regulation of energy metabolism.

Disclosures: **A. Stahr:** None. **M. Ingenwerth:** None. **E. Noichl:** None. **H. Korf:** None. **H. Reinke:** None. **C. von Gall:** None.

Poster

077. Thermoregulation and Energy Metabolism

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Topic: E.04. Autonomic Regulation

Support: R03-DA033453

Morell Dentistry Research Fund, UW

Title: Nitrous oxide evokes neuroendocrine stress and heat conservation responses

Authors: S. AL-NOORI[1], A. CIMPAN[2], Z. MALTZER[1], J. ZOU[3], K. J. KAIYALA[1], *D. S. RAMSAY[1];
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Abstract: Despite the extensive use of nitrous oxide (N2O) in dentistry and anesthesiology as well as its status as an abused inhalant, remarkably little is known about N2O's mechanisms of action. We hypothesized that as with many drugs of abuse, N2O activates neuroendocrine stress responses. As N2O also promotes hypothermia during initial administration, we hypothesized that the drug initially inhibits thermogenesis via brown adipose tissue (BAT) while promoting heat loss via the tail, major organs of thermoregulation in rodents. Finally, because N2O evokes hyperthermia following serial administrations, we hypothesized that elevated BAT thermogenesis plays a role in this thermoregulatory sign reversal. To assess hypothalamic-pituitary-adrenal (HPA) axis neuroendocrine responses and sympathetic autonomic neuroendocrine responses during initial N2O administration, we measured plasma corticosterone (CORT), norepinephrine (NEPI), and epinephrine (EPI) during an initial 2-h 60% N2O session (N=5 male rats, values are mean ± SE). CORT (ng/ml) increased from 38±25.3 at baseline to 528±174.5 during N2O (p=0.09). NEPI (pg/ml) increased from 237.0±34.9 at baseline to 387±42.2 (p=0.04). EPI (pg/ml) increased from 170.0±51.4 at baseline) to 316±132.2 during N2O (p=0.15). Accordingly, acute N2O administration appears to evoke classic neuroendocrine

stress responses. In a separate study, using dual probe telemetry implants and infrared (IR) thermography, we assessed the effects of nine repeated N2O administrations compared to control (con) administrations on core temperature (Tc), BAT temperature (Tbat) and tail temperature (Ttail). Telemetry revealed that both Tc and Tbat were reduced significantly during initial N2O administration (Tc: n=11 con, 12 N2O; N2O minus con difference averaged over 90 min (DT) = -0.5±0.12 C, p=0.001. Tbat: n=8 con, 8 N2O; DT = -1.1±0.18 C; p <0.001). IR thermography revealed that acute N2O administration unexpectedly inhibited Ttail (n=6 con, 7 N2O; DT = -1.6±0.36 C; p = 0.001). In the 9th session, N2O inhalation increased Tc (DT = 0.7±0.23 C; p = 0.007) indicative of a hyperthermic sign reversal, yet neither Tbat nor Ttail were different from control (Tbat: DT = 0.04±0.33 C; p = 0.9; Ttail: DT = 0.07±0.91 C; p = 0.9). Thus, acute 60% N2O evokes classical HPA and sympathetic neuroendocrine stress responses, and may promote hypothermia via reduced BAT thermogenesis accompanied by tail vasoconstriction as a compensatory mechanism to limit body heat loss. After repeated N2O administrations rats exhibit a hyperthermic Tc but a normalized Tbat, suggesting induction of a hyperthermia-promoting thermogenic adaptation of undetermined origin.

Disclosures: **S. Al-Noori:** None. **A. Cimpan:** None. **Z. Maltzer:** None. **J. Zou:** None. **K.J. Kaiyala:** None. **D.S. Ramsay:** None.

Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.07/U19

Topic: E.04. Autonomic Regulation

Support: NIH R15DK097644

AHA GIA 410805

Title: Predator odor rapidly increases skeletal muscle thermogenesis in rats

Authors: ***N. Y. MAVUNDZA**[1], M. E. SMYERS[2], R. M. CAMP[2], J. D. JOHNSON[2], C. M. NOVAK[2];
[1]Biomed. Sci., [2]Biol. Sci., Kent State Univ., Kent, OH

Abstract: Neural pathways in the brain integrate internal metabolic and external stimuli to effect behavioral and metabolic changes to optimize fitness. Hypothalamic pathways modulate metabolism of several peripheral systems including skeletal muscle through the sympathetic

nervous system. Activation of the ventromedial hypothalamus (VMH) is associated with increased muscle glucose uptake, insulin sensitivity, and fat oxidation. Although the VMH is widely known to integrate internal metabolic cues, external environmental stimuli also impact behavior and associated metabolic changes through their action on the VMH. The central/dorsomedial VMH is an integral part of the medial hypothalamic defense circuit and has recently been implicated in the behavioral and autonomic aspects of the predator response. Therefore, the overlap in structure suggests that there may be an interaction between these functions of the VMH - metabolic and defense. We have found an increase in skeletal muscle thermogenesis in rats following exposure to predator odor. This response was rapid, increasing muscle temperature 1° C by 2 min, and 1.8° C by 10 min after exposure to predator odor. It is unlikely that this was secondary to a general stress response because a shock stimulus induced a lower magnitude change in temperature. Predator odor - induced muscle thermogenesis is likewise not completely secondary to increased physical activity as the elevated muscle temperature persisted even when activity was held constant using a treadmill. Lastly, predator odor has the potential to significantly impact energy balance as energy expenditure was increased by 46% in the first hour after exposure. Based on these findings, we hypothesize that exposure to predator odor acts as an external environmental cue to optimize survival by activating not only the innate defense response but also coordinated metabolic and physiological responses.

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Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.08/U20

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH R15DK097644

AHA GIA 410805

R24 RR017718

ROD012098A

NIH RO1 DK077200

Title: Ventromedial hypothalamic melanocortin receptor activation differentially impacts skeletal muscle energetic pathways in lean vs. obesity-prone rats

Authors: *C. K. GAVINI[1], S. L. BRITTON[2], L. G. KOCH[2], C. M. NOVAK[1];
[1]school of biomedical sciences, Kent State Univ., Kent, OH; [2]Dept. of Anesthesiol., Univ. of Michigan, Ann Arbor, MI

Abstract: Rats selectively bred for high intrinsic aerobic capacity (HCR) are lean, resistant to obesity, and consistently more active than low-capacity runners (LCR), which are prone to metabolic syndrome. We have shown that HCR have elevated total daily energy expenditure (EE), primarily due to elevated physical activity (PA) plus low economy of activity in HCR wherein those excess calories are dissipated by skeletal muscle as heat. We focus on the ventromedial hypothalamus (VMH), part of a pathway regulating energy balance (EB) through its actions on peripheral glucose and lipid allocation, modulating respiratory quotient (RQ) and thermogenesis. The central melanocortin (MC) system also plays a vital role in modulating EB, increasing EE and PA, and decreasing appetite. We have demonstrated that activation of MC receptors in the VMH lowers RQ and increases activity EE both by increasing PA and lowering work efficiency, and that it more effectively impacts metabolism in HCR compared to LCR via differential activation of the sympathetic nervous system (SNS). Based on this, we hypothesize that MC receptor activation in the VMH will alter skeletal muscle metabolic pathways and this will be greater in HCR than in LCR. Male HCR/LCR rats (n=32; 8/group) with guide cannulae aimed at the VMH received microinjections of MC receptor agonist Melanotan-II (MT-II, 20pmoles/200nl) or vehicle (aCSF).We examined mRNA and protein expression of metabolic mediators in skeletal muscle, brown adipose, white adipose, and liver. Compared to vehicle microinjection, intra-VMH MT-II induced a significant increases or trends in mRNA expression of mediators of EE (UCPs, PPARs, PGC1a) with an increasing trend in protein expression; and a trend toward greater activation in HCR than in LCR. There were no significant changes in mediators of energy conservation (K+ATP channels, MED1). These results support the hypothesis that the brain-muscle pathway, modulated by MC receptors in the VMH, more effectively impacts metabolism in HCR compared to LCR, and may underlie the elevated activity EE in the lean phenotype.

Disclosures: **C.K. Gavini:** None. **S.L. Britton:** None. **L.G. Koch:** None. **C.M. Novak:** None.

Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.09/U21

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: Neuronal regulation of vitamin B12 metabolism under selenium-biased versus glutathione-biased redox conditions

Authors: *Y. LI[1], R. DETH[2];
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Abstract: Vitamin B12, also known as cobalamin (Cbl), is an essential micronutrient and neurologic dysfunction is a primary clinical manifestation of Cbl deficiency. Methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl) are active Cbl species, serving as enzyme cofactors, and it has been observed that the ability of cells to generate MeCbl and AdoCbl from other Cbl species depends on cellular redox status. The brain is present in a closed compartment surrounded by the blood-brain barrier with a unique redox environment. Both selenium-containing selenoproteins and glutathione (GSH) play important roles in the brain redox system. Availability of the GSH precursor cysteine is very limited in the brain, with a level in cerebrospinal fluid that is < 10% of the level in blood. Moreover, the transsulfuration pathway, which converts homocysteine to cysteine, is partially blocked in the brain. However, the brain exhibits a superior ability to retain and utilize selenium, which correspondingly compensates for the relative shortage of GSH. In order to investigate the effects of GSH-biased and selenium-biased redox conditions on neuronal cobalamin metabolism, we modified serum-free cell culture media with defined combinations of different cysteine and selenium concentrations to create GSH-biased vs. selenium-biased redox environments. Using the SH-SY5Y human neuroblastoma cell line as a neuronal cell model, we measured levels of individual Cbl species and a series of thiol metabolites with HPLC/electrochemical detection analysis. We found that the level of cyanocobalamin (CNCbl) was significantly decreased when both selenium and cysteine levels were low, suggesting that the cellular Cbl uptake was impaired, since CNCbl was the Cbl source in culture media. In addition, levels of both AdoCbl and MeCbl showed selenium and cysteine dependence, but cysteine in the culture media had a more profound effect on the level of MeCbl than selenium, which may be due to the fact that cysteine level could quickly and directly influence the level of GSH and subsequently affect formation of glutathionylCbl, a precursor of MeCbl. Consistent with this notion, the GSH level and the ratio of GSH to its oxidized form GSSG were more directly dependent on cysteine concentration than selenium concentration. To date there has been very little effort to identify the key brain-specific elements of antioxidant metabolism. Our findings begin to address this critical gap in knowledge and they indicate differential roles for GSH vs. selenoproteins in maintaining redox and MeCbl-dependent methylation status in the brain.

Disclosures: **Y. Li:** None. **R. Deth:** None.

Poster

077. Thermoregulation and Energy Metabolism

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Program#/Poster#: 77.10/U22

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant AG030399

NIH Grant AG037814

Title: Cerebral IL-17A improves glucose metabolism through AKT signaling

Authors: *J. YANG[1], J. KOU[1], J.-E. LIM[1], R. LALONDE[2], K.-I. FUKUCHI[1]; [1]Cancer Biol. & Pharmacol., Univ. of Illinois Col. of Med. At Peoria, Peoria, IL; [2]Dept. of Psychology, Univ. of Rouen, Rouen, France

Abstract: Interleukin-17A (IL-17A) is well known as a proinflammatory factor involved in many auto-immune diseases and metabolic disorders. Emerging studies have shown the connection between obesity and IL-17A in IL-17A knockout mice. However, the role of IL-17A in glucose metabolism is unclear. In this study, we found that both IL-17A and IL-17 receptor A are present in the mouse brain. Overexpression of IL-17A in the brains of mice via recombinant adeno-associated virus serotype 5 (rAAV5)-mediated gene delivery gave rise to loss of body weight and adipose tissue mass and an improvement in glucose tolerance and insulin sensitivity while overexpression of IL-17A in the skeletal muscles led to only a comparable improvement in glucose tolerance. Moreover, IL-17A enhances glucose uptake in PC12 cells by activation of AKT. Our results provide evidence for the first time that IL-17A in the brain plays a predominant role in regulating glucose metabolism, body weight and adipose tissue mass, particularly, enhancing glucose uptake by neurons via activation of AKT.

Disclosures: J. Yang: None. **J. Kou:** None. **J. Lim:** None. **R. Lalonde:** None. **K. Fukuchi:** None.

Poster

077. Thermoregulation and Energy Metabolism

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Natural Sciences and Engineering Research Council

Research Manitoba

St. Boniface Hospital Research Centre

Everett Endowment Fund

Title: NF-κB regulates neuronal bioenergetics *in vitro*

Authors: *W. SNOW[1,2], S. K. ROY CHOWDHURY[1], J. DJORDJEVIC[1], D. MCCALLISTER[1], C. CADONIC[1,3], P. FERNYHOUGH[1,2], B. C. ALBENSI[1,2,3];
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Abstract: Objective: Although considered important for immunity, the transcription factor nuclear factor kappa B (NF-κB) is also implicated in learning and memory. Mice lacking the p50 subunit of NF-κB display learning and memory deficits. NF-κB levels are altered after the induction of long-term potentiation (LTP), an experimental model of cellular learning and memory. Conversely, LTP is altered after blockade of NF-κB in hippocampal brain slices. In addition to synaptic plasticity, the regulation of neuronal bioenergetics is also central to learning and memory. NF-kB modulates cellular energy regulation (i.e., mitochondrial dynamics, glycolysis) in cancer biology. Whether NF-κB exerts similar influences on energy production in neurons, however, is not well established. NF-κB is present in the mitochondrial matrix in neurons. Further, the antioxidant manganese superoxide dismutase (MnSOD), localized to mitochondria, is a downstream target of NF-κB. This study investigated the effects of NF-κB blockade on cellular respiration and glycolysis in neuronal cultures as a means of investigating its role in neuronal energy homeostasis. **Methods:** Cortical neurons from embryonic CD1 mice (E15-16) were cultured in 24-well Seahorse (Seahorse Biosciences) culture plates (300,000/well). After 24-hr treatment with NF-κB inhibitors sulfasalazine and SN50 at DIV 8-9, oxygen consumption rates (OCR) and glycolysis (extracellular acidification rates; ECAR) were measured in real time using the XF24 Analyzer (Seahorse Biosciences) and compared to untreated controls. Western blots were used to detect levels of NF-κB and MnSOD after NF-κB blockade. **Results:** Treatment (24 hr) with 1 mM sulfasalazine decreased maximal respiration ($p<0.05$) in neurons. SN50 decreased maximal respiration rates in a dose-dependent fashion ($p<0.05$). Sulfasalazine had no effect on ECAR at baseline. Glycolysis, however, was diminished in neurons treated with varying doses (300 uM-1 mM) of sulfasalazine ($p<0.001$), as was glycolytic capacity ($p<0.01$). Western blot experiments are ongoing. **Conclusions:** These data establish a novel role for NF-κB in neuronal bioenergetics. Such results have important implications for the treatment of disorders in which brain energy regulation and memory are compromised, including Alzheimer's disease. **Acknowledgement:** Funding from Natural

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Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.12/U24

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NINDS

Title: Modulation of neuronal mitochondrial motility and calcium responses by solutions enriched with oxygen nanobubbles

Authors: ***M. V. IVANNIKOV**, M. SUGIMORI, R. LLINAS;
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Abstract: Mitochondrial motility is believed to be driven by changing ATP needs in spatially segregated neuronal domains, and regulated by increases in cytosolic calcium levels. In previous studies we showed that treatments with solutions enriched in oxygen nanobubbles (RNS60, ~ 55±5 ppm O_2), as opposed to hyperbaric oxygen solutions (ONS60, ~ 55±5 ppm O_2), lead to significant increases in neurotransmitter release in the squid giant synapse (Choi et al., 2014) and in mouse diaphragm muscle preparations (Walton et al., 2014). These were both accompanied by increases in cellular ATP levels. Moreover, such changes could be completely abolished by mitochondrial inhibitors (Choi et al., 2014). Our preliminary data in differentiated PC12 and cultured cerebellar granule neurons show that the rise in cytosolic ATP levels with RNS60 leads to increased mitochondrial motility. Mean mitochondrial velocity in RNS60 treated cells was 0.31±0.05 um/sec (n=12) compared to 0.17±0.03 um/sec (n=12) in controls at room temperature. Additionally, the cytosolic area affected by mitochondrial motility measured over 10 min time period was also significantly increased by RNS60. RNS60 did not affect resting cytosolic calcium levels measured with fura-2AM in cerebellar granule neurons. However, the amplitude of cytosolic calcium transients evoked by brief applications of 60 mM potassium chloride Tyrode's solutions in fluo-4AM loaded cerebellar granule neurons was increased by 16.3± 4.8 % (n=6). Taken together, these findings suggest that stimulation of mitochondrial ATP production

with oxygen nanobubbles likely leads to a partial dissipation of intracellular ATP and ADP gradients despite increased cytolic calcium influx, which disinhibits mitochondrial motility.

Disclosures: **M.V. Ivannikov:** None. **M. Sugimori:** None. **R. Llinas:** None.

Poster

077. Thermoregulation and Energy Metabolism

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH/NIA AG043972

UofMN Medical School and Minnesota Medical Foundation

graduate student fellowship of the University of Parma

Title: Mouse chronic subordination stress to model eating disorder-based metabolic dysfunctions

Authors: ***M. RAZZOLI**[1], V. SANGHEZ[2], A. BARTOLOMUCCI[1];
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Abstract: Eating disorders and their physical and psychiatric co-morbidities appear to have causal factors including stressful life events and negative affect. By far the most common, binge eating disorder (BED) is defined by eating in a discrete period of time a larger than normal amount of food, a sense of lack of control over eating, and marked distress. Its underlying mechanisms are still unknown, in part due to the lack of appropriate animal models. We developed a naturalistic murine model of chronic subordination stress (CSS)-induced hyperphagia linked to metabolic disturbances. Subordinate male mice become hyperphagic and vulnerable to weight gain, show high leptin, low adiponectin, dyslipidemia and tissue-specific down-regulation of the insulin and peroxisome proliferator activated receptors pathways, indicative of insulin resistance. Furthermore, when high fat diet is allocated to hyperphagic subordinate mice their metabolism is further aggravated to pre-diabetes-like state. Since subordinate hyperphagic behavior is at the core of the described metabolic phenotype we sought to characterize it towards BED. In dedicated experiments, CSS was associated to the automated acquisition of subordinate food intake for meal pattern analysis. Additionally, we tested the hypothesis that the manifestation of obesity and metabolic syndrome could be prevented by limiting hyperphagia applying a pair-feeding protocol to subordinate mice. CSS disrupted the

architecture of feeding: subordinate mice ingested a disproportionate amount of food at higher rate and with shorter satiety ratio than control mice, in analogy to BED diagnostic criteria. Either hunger or acute stress were able to further exacerbate subordinate mice hyperphagia, suggesting a stress over-reactive state. Notably, preventing hyperphagia by pair-feeding abrogated the obese phenotype but not the fasting hyperglycemia of subordinate mice, probably due to a greater hunger-stress sensitivity. Overall these results support the validity of mouse CSS to model binge eating disorder addressing the contribution of the individual socioeconomic status on health in the development of eating disorders and their metabolic sequelae. Further characterization of the subordinate phenotype will help identify the molecular mechanisms involved in the regulation and the control of food intake in a psychologically and metabolically challenging context to generate knowledge-based innovative therapies.

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Poster

077. Thermoregulation and Energy Metabolism

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: T32-HD05785

KUMC School of Health Professions

Title: Behavioral, metabolic, and iron defects associated with loss of Ncb5or in the mouse cerebellum

Authors: *M. A. STROH;
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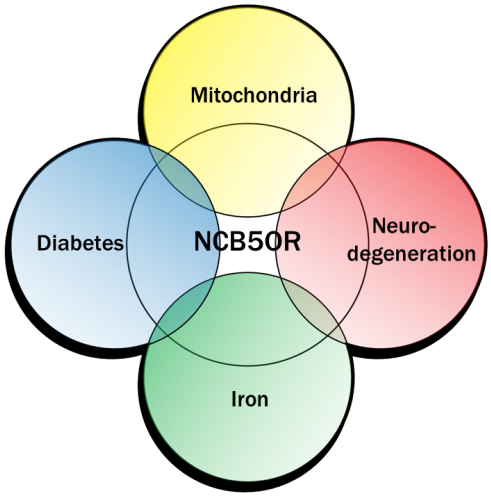
Abstract: Loss of NADH cytochrome b5 oxidoreductase (Ncb5or), a ubiquitously expressed flavoheme oxidoreductase, has been shown to result in pancreatic beta cell death, increased oxidative stress, altered lipid metabolism, as well as iron dyshomeostasis, hypermetabolism, and hyperactivity in mice. We recently conducted radioactive iron (Fe-59) pulse feeding experiments on global knockout (KO) mice and found that, while total brain iron content was unchanged, the amount of radioactive iron present in the brains of KO mice was significantly increased, suggesting an increased iron flux. To further investigate the effects of Ncb5or deficiency on neural tissue we generated a conditional knockout (CKO) mouse that lacks NCB5OR in the

cerebellum. Early longitudinal behavior tests reveal hind-limb clasping associated with changes in proprioceptive processing as early as 3 weeks of age in CKO mice. At 7 weeks of age, dietary iron deficiency results in alterations in locomotion and coordination in both wild type and CKO mice. However WT and CKO mice display opposite trends, with WT mice becoming hyperactive and CKO mice becoming hypoactive compared to age and genotype matched chow fed mice. Assessment of molecular markers in the cerebellum of CKO mice at age 7 weeks reveals changes in transcript levels in many genes associated with the maintenance of iron homeostasis as well as iron-sulfur containing proteins of the mitochondrial electron transport chain. Finally, intraperitoneal injection of 14C labeled 2-deoxyglucose revealed a significantly increased uptake of 2-deoxyglucose in the cerebellum of CKO mice as early as 7 weeks of age. Taken together, these findings suggest an important role for Ncb5or in maintaining iron homeostasis as well as potential influence over metabolic processes. A longer longitudinal study is currently underway to determine the behavioral effects of Ncb5or deficiency during brain aging.



Disclosures: **M.A. Stroh:** None.

Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.15/U27

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH-P50 (81754)

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Shriners Hospitals for Children (84090, 71006, 85310)

American Diabetes Association (67666)

Title: Contribution of centrally acting hormones amylin and ghrelin on the adipose tissue transformation in burn victims

Authors: *M. K. SARAF[1,2], D. N. HERNDON[2], C. PORTER[2], R. RADHAKRISHNAN[3], M. CHONDRONIKOLA[3], T. CHAO[3], L. S. SIDOSSIS[2];
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Abstract: Severe burn injury causes massive metabolic abnormalities including catecholamine surge, lipolysis, and hormonal imbalance in burned children. We have recently demonstrated morphological changes and increased UCP1 expression in subcutaneous white adipose tissue (WAT) of human burn survivors (i.e. browning of WAT). Rodent studies suggest that amylin and ghrelin may be involved in the regulation of thermogenesis and energy expenditure, possibly by triggering sympathetic nerve activity innervating WAT. This study investigated the association between amylin and ghrelin hormones and the browning of WAT in burn victims. Subcutaneous WAT and blood samples were collected from twelve burned children (13±6yrs; 57% total body surface area burned) and nine healthy children (9±5 yrs). Hormones were measured by 'magnetic bead panel based multiplex' assay kit. Histology, immunohistochemistry, RT-PCR and mitochondrial respiration measurements were performed in WAT. Higher UCP1 mRNA ($p < 0.01$), UCP1 protein (22.9±4.5ng/mg vs 2.7±0.3; $p<0.001$), UCP1 immuno-staining, interleukin-6 (1307±282pg/ml vs 36±24; $p<0.001$) and mitochondrial respiration (1.5±0.3 vs 0.6±0.1 pmol/sec/mg, $p<0.05$) of burn WAT compared to healthy controls, indicates browning of WAT. Tissue norepinephrine level (53±20 pg/mg vs 15.2±10.2 pg/mg) was increased in WAT of burn victims after two week of post burn as compared to early days, indicating persistent adrenergic stimulation. We found high amylin (123.6±18pg/ml vs 24.7±4.6 pg/ml; $p<0.01$) and low ghrelin (15.2±3.5 pg/ml vs 27.8±7.3; $p<0.05$) level in plasma of burn victims. Amylin levels were significantly correlated with mitochondrial respiration ($R2=0.53$, $p<0.05$). Hence we conclude that centrally acting amylin and ghrelin may contribute, at least in part, to the WAT transformation.

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Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

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Program#/Poster#: 77.16/U28

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH MH087978

NIH MH100828

NIEHS ES019851

Title: The effect of early life methyl donor supplementation on obesity development

Authors: *S. E. MCKEE[1], T. M. REYES[2,1];
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Abstract: Nearly 50% of women with a normal pre-pregnancy body mass index gain more weight than recommended during their pregnancy. This excessive gestational weight gain (GWG) significantly increases the risk for the development of obesity in the offspring. Excessive GWG can be modeled in animals by feeding pregnant dams a high fat diet, leading to increased maternal weight gain and obesity-promoting phenotypes in the offspring. Using a mouse model of excessive GWG, we have documented in the offspring an increased preference for sucrose and fat, increased expression of genes that underlie reward-related behaviors, and both global and gene specific decreases in DNA methylation. These changes in reward-related neural circuitry may contribute to the increased risk for the development of obesity in the offspring by altering the animal's response to highly palatable, energy dense foods. Methyl donor supplementation (MDS) during pregnancy can reverse some of these obesity-promoting phenotypes in offspring, yet it is unknown whether postnatal MDS can reverse these phenotypes. To determine this, offspring from dams fed either a high fat diet (HFD) or control diet during gestation were fed a methyl donor supplemented diet during early life (3-6 weeks). We find that postnatal MDS significantly decreased body weight in both male and female adult HFD offspring, and does not alter body weights of control diet offspring. Further, postnatal MDS can normalize adult male fat preference and contributes to region specific normalization of DNA hypomethylation. Continued assessment of obesity development, preference for palatable foods, metabolic phenotypes, and expression changes of genes underlying homeostatic and hedonic feeding will elucidate whether early postnatal nutritional interventions can be utilized as a therapy to offset adult obesity risk.

Disclosures: **S.E. McKee:** None. **T.M. Reyes:** None.

Poster

077. Thermoregulation and Energy Metabolism

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.17/U29

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: British Heart Foundation

Title: Fast-cyclic voltammetry reveals altered oxygen homeostasis in the nucleus tractus solitarii of the spontaneously hypertensive rat

Authors: *P. S. HOSFORD[1], J. MILLAR[2], A. G. RAMAGE[1], A. V. GOURINE[1], N. MARINA[1];
[1]Univ. College, London, London, United Kingdom; [2]QMUL Sch. of Med. and Dent., London, United Kingdom

Abstract: We have recently shown that in spontaneously hypertensive rats (SHRs) pO_2 of the brainstem parenchyma is lower compared to that of normotensive animals. We hypothesized that in conditions of decreased pO_2 astrocytes release ATP and L-lactate which contribute to the development of arterial hypertension by increasing activity of pre-sympathetic circuits (Marina et al., 2015). In this study, we investigated pO_2 homestasis in a brainstem area important for the control of blood pressure. We used fast-cyclic voltammetry to detect real time changes in tissue pO_2 in the nucleus tractus solitarii (NTS) in response to electrical simulation of the vagus nerve in α-chloralose anesthetized SHR (n=7) and Wistar (n=6) rats. Resting parenchymal pO_2 was determined in the NTS at natural arterial blood pressure (BP) for both strains (SHR; BP ~150, Wistar; ~100mmHg) and was found to be significantly ($P<0.05$) lower in the SHRs (51±4 vs 71±6 mmHg). Electrical stimulation of the central end of the vagus nerve (0.8mA, 1ms, 20s) caused a decrease in blood pressure and heart rate accompanied by a biphasic change in tissue pO_2 characterized by an initial decrease followed by an 'overshoot' above the baseline value. The initial pO_2 decrease was found to be significantly smaller in the SHRs compared to Wistar rats when stimulated at 3Hz (-5 ± 3 vs -18 ± 6 mmHg) and 5Hz (-7±3 vs -23 ±3 mmHg), respectively. However, there was no difference in the amplitude of the pO_2 overshoot. Animals subsequently received a ganglionic blocker (chlorisondamine, 1mg kg^{-1}) and mean arterial BP was clamped at 100mmHg by phenylephrine infusion (~20μg min^{-1}). In these conditions blood pressure and heart rate changes evoked by vagal stimulation were abolished; the initial decrease in tissue pO_2 was still significantly smaller in SHRs compared to Wistars when stimulated at 5Hz (-1 ± 1 vs -11 ± 4 mmHg) and 10Hz (-6 ± 2 vs -17 ± 5 mmHg). However, pO_2 overshoot became significantly higher in SHRs when stimulated at 5Hz (14 ± 2 vs -8 ± 1mmHg) and 10Hz (12 ±1

vs 6 ± 3 mmHg). In summary, at the normal level of the arterial BP the NTS of SHRs display smaller decreases in tissue pO_2 in response to increased neuronal activity but larger overshoots than Wistars. Further, SHR have a lower resting parenchymal pO_2. The evidence obtained indicates that oxygen homeostasis is altered in the SHR. The profile of pO_2 changes triggered by enhanced neuronal activity is also different which may reflect differences in vascular anatomy and/or perfusion between the two strains.

Disclosures: P.S. Hosford: None. **J. Millar:** None. **A.G. Ramage:** None. **A.V. Gourine:** None. **N. Marina:** None.

Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.18/U30

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: R15 403009

RO1 443163

R24 OD010950

Title: Changes in brain melanocortin system with calorie restriction-induced adaptive thermogenesis and suppressed physical activity

Authors: *S. MUKHERJEE[1], S. L. BRITTON[3], L. G. KOCH[3], C. M. NOVAK[2];
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Abstract: Obesity, a growing problem in today's society, can be reversed by energy restriction, but this causes metabolic compensations hampering continued weight loss. During weight loss, energy expenditure (EE) is suppressed beyond what is predicted by reduced body weight, a phenomenon termed adaptive thermogenesis. For our experiments we used rats artificially selected for high and low intrinsic aerobic capacity (HCR, LCR) which respond differently to 50% calorie restriction (CR), specifically with respect to suppression of physical activity and activity-related EE. Here, we examined changes in the brain melanocortin (MC) system over 21 days of calorie restriction in male HCR and LCR. In the arcuate nucleus (ARC), opposing anorexigenic POMC/CART (proopiomelanocortin/cocaine- and amphetamine-regulated transcript) neurons and orexigenic NPY/AgRP (neuropeptide Y and agouti related-peptide)

neurons modulate energy balance homeostasis, partly through neuropeptide actions on MC receptors. To determine if this system changes in association with adaptive thermogenesis, micropunching was employed to extract hypothalamic regions including the ARC, paraventricular nucleus (PVN), perifornical lateral hypothalamus (PeFLH), dorsomedial hypothalamus (DMH), and ventromedial hypothalamus (VMH) from frozen brain sections. QPCR was performed to examine the RNA expression of the melanocortin receptor subtypes 3, 4, 5 (MC3R, MC4R, MC5R). Comparisons were made between HCR and LCR at ad libitium feeding and after 50% CR at 2 days and 21 days. Expression of MC3R in the PVN decreased in HCR at 21-day CR. This is consistent with our findings demonstrating that MCR activation in the PVN promotes physical activity, and that physical activity decreases in HCR (but not LCR) after 21 days of CR. Changes were also seen in MC4R in VMH, where HCR increased expression throughout the course of CR. Whereas for MC5R there was a significant interaction between HCR and LCR in the ARC where HCR showed an increase, but LCR showed a decrease, after 2 days of CR. AgRP expression increased in both HCR and LCR by 21-day CR. Thus, just as energy restriction results in different degrees of activity-associated adaptive thermogenesis and physical activity suppression in HCR vs. LCR, we also find that 21-day CR differentially affects some, but not all, aspects of the brain MC system. These changes may result from opposing effects of CR on the two peptides-melanocortins and AgRP-that act on the same receptors, suggesting a possible role of orexigenic AgRP in changes in activity during energy restriction, especially long-term restriction.

Disclosures: **S. Mukherjee:** None. **S.L. Britton:** None. **L.G. Koch:** None. **C.M. Novak:** None.

Poster

077. Thermoregulation and Energy Metabolism

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: FC-2014

Scholarship-2014 to DJBV by SEP

Title: Metabolic glucose, insulin and leptin circadian rhythms are altered by perinatal cafeteria diet in rats

Authors: D. J. BUSTAMANTE-VALDEZ, *P. DURAN;
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Abstract: The SCN receives information of the metabolic status of the body using input from the paraventricular nucleus,as well it is known to be the master clock in mammals.The food intake in quantity, quality and time is related to the correct use of metabolic hormones, and in the energy balance. Proper nutrition is one of the factors involved in the health and development of animals. The quantity and quality of the nutrients must satisfy the vital needs of the individuals, so that an imbalance in these causes changes at different levels which are hormonal, metabolic and organizational into the nervous system, depending on the moment in which it is established, can influence in the critical periods of brain and somatic development in the short, medium- or long-term. The aim of the present study, was to evaluate and to establish differences in daily profiles as well as in the rhythm amplitude and acrophase of plasma glucose, insulin, and leptin concentrations, levels of corticosterone were also evaluated as a control to stressors and circadian profile of a non-metabolic hormone in hypercaloric malnourished juvenile male rats. Sprague-Dawley female rats were randomly divided into 2 nutritional protocols (control -CO and high calorie/low protein malnutrition-MHp) with water and food ad libitum, three week prior the mating and through the gestation and lactation periods. The postnatal development of the offspring was observed, and pups from MHp group showed a decrease in size and weight, as well as an area significantly minor under the curve on the glucose tolerance test. Analysis of plasma glucose, insulin, and leptin parameters threw an imbalance in energy metabolism during 24 hours, a Cosinor test was performed to evaluate the daily rhythms insulin and leptin which occurred in CO but not in MHp group, glucose 24hr rhythms was present in both groups. We found a difference in the model of homeostatic assessment (HOMA) over 24hr, HOMA-IR (insulin resistance), was significantly greater in ZT4 and ZT20 of CO compared to MHp. HOMA-B (beta pancreatic cells) was significantly higher in ZT4 and ZT16 in MHp vs CO. Therefore it can be inferred that the disparity presented in MHp compared to CO in the daily rhythm of glucose, insulin and leptin, as well as the delay in growth are due to perinatal exposure of the high calorie/low protein diet known as “cafeteria diet”. Nutritional conditions during early stages of development have an influence on the energy balance in later stages, a disparity in the daily rhythmicity of glucose, insulin and leptin can be related to conditions or diseases at later stages.

Disclosures: D.J. Bustamante-Valdez: None. **P. Duran:** None.

Poster

077. Thermoregulation and Energy Metabolism

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: JSPS Grant

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MEXT Grant for the the Body and Mind Integrated Sports Sciences (BAMIS) project

MEXT Grant for for the Human High Performance (HHP) project

Title: Brain glycogen fuels the exercising brain to maintain endurance capacity

Authors: *T. MATSUI, H. OMURO, Y.-F. LIU, T. SHIMA, M. SOYA, M. HAMASAKI, S. MIYAKAWA, H. SOYA;
Univ. of Tsukuba, Ibaraki, Japan

Abstract: During exercise, energy supply for active organs such as skeletal muscles and brains is essential issue to maintain endurance capacity. Although muscle glycogen, the glucose storage molecule, is the essential energy source in exercising muscles, energetics in the exercising brain is still largely unknown. Since brain glycogen stored in astrocytes, a source of lactate as a neuronal energy source, decreases during prolonged exhaustive exercise, we hypothesized that brain glycogen plays an important role in exercise endurance through energetic contributions in the exercising brain. To test this hypothesis, we here employed a rat model of prolonged exhaustive exercise on the treadmill with high-power microwave irradiation (10 kW, 1.2 sec), which is a sacrificing method as the gold standard for detecting brain metabolites including glycogen *in vivo*. Prolonged exhaustive exercise increased blood lactate levels and induced hypoglycemia, muscle glycogen depletion, and decreases, but not depletions, in brain glycogen levels together with increased neuronal monocarboxylate transporter (MCT2) protein levels. At the end of exhaustive exercise (time to exhaustion, 84.4 ± 2.9 min), metabolomics revealed increases in brain lactate levels, but not in muscle, with sustained concentrations of glycolytic sources. Simultaneously, brain ATP levels were maintained, while muscle ATP levels was depleted. Last, blockade of brain glycogenolysis and MCT2 during exercise by icv-injection with 1,4-Dideoxy-1,4-imino-d-arabinitol (DAB) and α-cyano-4-hydroxy-cinnamate (4-CIN), respectively, decreased endurance capacity associated with brain ATP levels. Our findings demonstrated for the first time that lactate from brain glycogen fuels the exercising brain to retain endurance capacity, providing a new understanding of brain energetics during exercise. Residual brain glycogen is a potential defense mechanism for maintaining brain ATP during exhaustive exercise.

Disclosures: **T. Matsui:** None. **H. Omuro:** None. **Y. Liu:** None. **T. Shima:** None. **M. Soya:** None. **M. Hamasaki:** None. **S. Miyakawa:** None. **H. Soya:** None.

Poster

077. Thermoregulation and Energy Metabolism

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 77.21/U33

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH2R15NS060117-02

Title: Role of TRPV4 in prediabetic obese peripheral nerve

Authors: C. AVOUNDJIAN, B. COOPERMAN, *L. R. BANNER;
Biol., California State Univ. Northridge, Northridge, CA

Abstract: Overconsumption of food and inactivity on a regular basis are risk factors for developing prediabetic obesity. According to the American Diabetes Association, prediabetes affects over 86 million Americans which, if not taken care of, may cause type II diabetes. This disease state is characterized by higher than average blood sugar levels and depending on the severity, may lead to a number of problems in immune and nervous systems. Although studies are limited, prediabetes has been shown to induce complications in the peripheral nervous system (PNS) - a common complication in diabetic patients. Prediabetic neuropathy causes differences in the conduction of motor and sensory nerves as well as abnormalities in nerve function. Transient Receptor Potential Vanilloid 4 (TRPV4), is a cation channel responsible for sensing a variety of stimuli and is found within the spinal cord and sensory neurons of the peripheral nervous system. Recent studies have demonstrated a role for TRPV4 in regulating peripheral neurite growth. The expression of TRPV4 in prediabetic peripheral nerves may play a role in establishing differences in the nerves of prediabetic obese and normal mice. In order to examine the effects of TRPV4 in the sciatic nerves of prediabetic obese mice, we measured the quantity of the ion channel present within the nerves of high fat fed and control fed mice. Six-week-old C57BL6/J bred mice were placed on a 60% high fat or 10% control diet for 19 weeks. Blood glucose and weight measurements were then used to determine prediabetic condition. Animals fed a high fat diet weighed significantly more and displayed elevated blood glucose levels than mice fed the control diet. Sciatic nerves from these animals were extracted for protein, quantified with the BCA kit, and used for Western blot analysis. Our preliminary analysis revealed higher TRPV4 levels in the sciatic nerves of diet induced obese mice relative to the levels of TRPV4 in control fed mice. This suggests that the upregulation of TRPV4 produced from a high fat diet may have an effect on prediabetic nerve function. Nerve conductions studies will be performed following agonist and antagonist application to address the role of TRPV4 in diet-induced prediabetic neuropathy.

Disclosures: C. Avoundjian: None. **B. Cooperman:** None. **L.R. Banner:** None.

Poster

078. Motor and Sequence Learning

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 78.01/U34

Topic: F.01. Human Cognition and Behavior

Support: R01 NS084948

Title: Implicit motor learning in the absence of sensory-prediction errors

Authors: *D. GRAEUPNER[1], P. A. BUTCHER[1], J. A. TAYLOR[2];
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Abstract: Recent studies have shown that learning in visuomotor adaptation tasks results from the interplay between implicit learning of a forward model, driven by sensory-prediction errors, and explicit learning, driven by target errors (Taylor, Krakauer & Ivry, 2014). Sensory-prediction errors are thought to be dependent on cursor feedback, while target errors are thought to be a proxy of a reward signal; however, the relationship between these two error sources with respect to implicit and explicit learning remains unclear. The goal of the current study was to systematically manipulate cursor feedback and reward feedback, and measure their relative contribution to implicit and explicit learning. Here, we employed a 2-by-2 factorial design, manipulating type of error signal (Endpoint vs. Scaled reward) and number of training targets (One vs. Eight) in a visuomotor rotation task. Explicit and implicit learning were distinguished by asking participants to verbally report their intended aiming direction on each trial. In the Endpoint conditions, error feedback was given in the form of a red circle indicating hand position, while in the Scaled reward conditions, error feedback was delivered as points (0-100) indicating the distance between an unseen cursor and the target. Additionally, we varied the number of target locations in each group: using either One or Eight targets. For the Eight target conditions, the targets could appear at one of eight locations that were separated by 45° along a numbered ring. We found that participants in all conditions learned to counter the rotation to a similar degree. Participants in the Endpoint feedback conditions showed similar traces of explicit and implicit learning, although, consistent with prior work from our lab, their relative contribution differed with respect to the number of targets. Surprisingly, implicit learning was also present in the Scaled reward feedback condition when training was limited to one target. In fact, here the time course of implicit learning was similar between Endpoint and Reward feedback conditions. When training at eight targets with reward feedback, implicit learning was greatly diminished. These findings suggest that the time course of implicit learning is itself

composed of multiple processes that evolve over the course of training in a similar manner. However, reward feedback is not sufficient to induce implicit learning when training throughout the workspace. In summary these findings indicate that implicit learning under reward feedback may be consistent with operant conditioning or use-dependent plasticity as opposed to a forward model.

Disclosures: D. Graeupner: None. **P.A. Butcher:** None. **J.A. Taylor:** None.

Poster

078. Motor and Sequence Learning

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 78.02/U35

Topic: F.01. Human Cognition and Behavior

Support: NSF

Title: Modifying the discrete sequence production task for a multi day tdcs study in young and older adults

Authors: *B. GREELEY[1,2], J. BARNHOORN[5], W. VERWEY[5], R. SEILDER[1,2,3,4];
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Abstract: The discrete sequence production (DSP) task is an explicit motor learning sequence task where two 6-item sequences are presented one item at a time. Over many repetitions, participants eventually execute a 6-item sequence as 2 or more segments, an indication of distinct motor chunks. Previous work has demonstrated that older adults exhibit a reduction in chunk length and have an impaired explicit memory, relative to young adults. Right and left dorsolateral prefrontal cortex (DLPFC) have been demonstrated to be involved in early explicit sequence learning as well as early adaptation. Primary motor cortex (M1) has been shown to be involved in explicit sequence learning and retention. Further, premotor cortex has been shown to be involved in memory consolidation in sequence learning. Here, we use transcranial direct current stimulation (tDCS), a non-invasive form of brain stimulation, to facilitate early learning and chunking in both younger and older adults in a truncated version of the traditional DSP task. Participants attend three sessions over the course of a week, and are randomized into one of five tDCS conditions (right DLPFC, left DLPFC, M1, premotor, or sham). Over the three sessions, participants complete a battery of cognitive and motor tasks that correlate with motor learning ability and executive functioning in order to characterize the participant, use later as covariates in

analysis, and understand how these cognitive and motor tasks might change from baseline as a function of the tDCS condition. Participants also practice the DSP task while receiving tDCS for up to 25 minutes during sessions 1 and 2. During session three, participants are tested on their ability to remember the sequence of the DSP task without stimulation. We hypothesize that tDCS to right DLPFC will facilitate early learning in both older and younger adults, with older adults receiving the most benefit from the tDCS stimulation. We also predict that tDCS over premotor cortex will help facilitate chunking in older adults, relative to older adults in the sham group. We expect that stimulation to M1, left DLPFC, and premotor in younger adults will change the rate of motor learning relative to young adults in the sham tDCS group. Our preliminary results suggest that younger adults without tDCS are still able to chunk with fewer trials in the DSP task over three sessions.

Disclosures: **B. Greeley:** None. **J. Barnhoorn:** None. **W. Verwey:** None. **R. Seilder:** None.

Poster

078. Motor and Sequence Learning

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Program#/Poster#: 78.03/U36

Topic: F.01. Human Cognition and Behavior

Support: VO 1432/15-1

Title: Fine motor control is associated with individual fitness level in older adults

Authors: *C. VOELCKER-REHAGE[1,2], L. HUEBNER[1,2], B. GODDE[1];
[1]Jacobs Univ. Bremen, Bremen, Germany; [2]Technische Univ. Chemnitz, Chemnitz, Germany

Abstract: A physically active lifestyle positively influences cognitive performance as well as corresponding brain structures and functions in elderly persons (e.g., Colcombe & Kramer, 2003). Recent studies showed a positive influence of a high fitness level on motor performance (e.g., Claudino et al., 2013). Fitness level was assessed by a physical activity questionnaire. The aim of the current study was to explore whether high fit older adults exhibit better performance than low fit participants also in a visuomotor tracking task requiring fine motor control, when fitness status is quantitatively measured with a cardiovascular exercise test. Data were collected from 52 older adults ranging from 67 to 83 years of age. Participants were assigned into two extreme groups based on their VO_2-peak from a cardiovascular spiroergometry on a bicycle ergometer (low fit group: VO_2-peak $\leq$ 1.10 l/min, n = 12; high fit group: VO_2-peak $\geq$ 1.50 l/min, n = 9). Fine motor control was assessed with a visuomotor precision grip tracking task.

Participants had to track two different irregular sine wave patterns with their right dominant hand (16 trials of 15 seconds each). Tracking variability was operationalized by use of the root mean square error (RMSE). All participants completed the Montreal Cognitive Assessment (MoCA) to control for cognitive decline (scores ranging from 21 to 30). A significant correlation of MoCA score and fine motor performance was found (r = -.035, p = .011), therefore further analyses were controlled for MoCA score (ANCOVA: 2 fitness (low fit, high fit) × fine motor control performance (RMSE) with MoCA score as covariate). High fit older adults performed better in the visuomotor tracking task revealed by a smaller RMSE (F (1, 20) = 5,476, p = .031, η^2_p = .233) as compared to the low fit group. MoCA score was significant (F (1, 20) = 7.600, p = .013, η^2_p = .297) indicating a moderating effect of cognitive performance on fine motor control. These results indicate that the performance in our visuomotor tracking task is, besides cognitive status, positively influenced by a high fitness level. Thus fitness might compensate not only for cognitive, but also for manual decline. Currently, analysis of corresponding EEG data is in progress. We expect to find supporting evidence by differences in electrophysiological correlates of high and low fit older adults. Claudino, R., Mazo, G. Z., & Santos, M. J. (2013). Age-related changes of grip force control in physical active adults. *Perceptual & Motor Skills, 116*(3), 859-871. Colcombe, S., & Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: A meta-analytic study. *Psychological Science, 14*(2), 125-130.

Disclosures: C. Voelcker-Rehage: None. **L. Huebner:** None. **B. Godde:** None.

Poster

078. Motor and Sequence Learning

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 78.04/U37

Topic: F.01. Human Cognition and Behavior

Support: Volkswagen Foundation II/83 177

Title: Motor plasticity in assembly-line workers: effects of repeated work task changes on manual dexterity and related brain function

Authors: *B. GODDE[1], J. OLTMANNS[1], C. VOELCKER-REHAGE[1], U. M. STAUDINGER[2]; [1]Jacobs Univ., Bremen, Germany; [2]Columbia Aging Ctr., New York, NY

Abstract: Work demands have a strong influence on brain and cognition. It has been assumed that particularly cognitive challenges in confrontation with unknown problems and novel situations facilitate positive plasticity in adult development [1,2]. We recently provided evidence

that also repeated confrontation with novelty at work at low levels of job complexity (indicated via repeated work-related task changes; WTC) positively affects cognitive flexibility and brain structure in adults across the working life span [3]. Here we were interested whether repeated WTC facilitate motor plasticity in long-term assembly-line workers. Of 3.500 workers from a production company in northern Germany who had been full-time employed with that company over the last 16 years, 179 persons returned a screening questionnaire. This allowed us to identify 10 (n=20) pairs of participants who differed in WTC (high/low) but were optimally matched for age, sex, job complexity as well as academic performance, openness to new experience and leisure time activity in young adulthood. In order to investigate long-term effects of WTC on manual dexterity and related brain function, we compared functional MR images from the participants while they performed a manual visuomotor force tracking task. First results revealed that participants with high WTC did not differ in motor performance levels and learning slopes from their low WTC matched counterparts. However, similar performance was associated with different brain activation patterns. While high WTC participants revealed focal activations in contralateral sensorimotor and parietal brain areas, low WTC was associated with much more distributed and diffuse activations in bilateral, also frontal brain areas. Interestingly, activation patterns found in low WTC participants resembled those previously found in older as compared to younger adults. Thus we conclude that repeated WTC facilitate motor plasticity in construction workers and might delay aging of the motor system. References: [1] Bowen CE, Noack CMG, Staudinger UM (2010). Aging in the work context. In K. W. Schaie & S. Willis (Eds.), Handbook of the psychology of aging (7th ed., pp. 263-277). San Diego, CA: Elsevier Academic Press. [2] Lövdén M, Backman L, Lindenberger U, Schaefer S, Schmiedek F (2010). A theoretical framework for the study of adult cognitive plasticity. Psychological Bulletin, 136, 659-676. [3] Godde B, Oltmanns J, Staudinger UM (2014) Don’t Lose Your Brain at Work - Work Task Mobility is Associated with Greater Brain Volume in Frontal and Striatal Regions. Program No. 840.01. 2014 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2014. Online.

Disclosures: **B. Godde:** None. **J. Oltmanns:** None. **C. Voelcker-Rehage:** None. **U.M. Staudinger:** None.

Poster

078. Motor and Sequence Learning

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 78.05/U38

Topic: F.01. Human Cognition and Behavior

Support: DFG GO-8 Re-LOAD

Title: Task-related alpha power during a fine motor control task in young and older adults

Authors: *L. HUEBNER[1,2], B. GODDE[1], C. VOELCKER-REHAGE[1,2];
[1]Jacobs Univ. Bremen, Bremen, Germany; [2]Technische Univ. Chemnitz, Chemnitz, Germany

Abstract: Task-related alpha power over sensorimotor and parietal areas decreases during the execution of a motor task (Manganotti et al., 1998). Others reported that older adults require increased prefrontal resources during motor control tasks (Berchicci et al., 2012). This study aimed to investigate whether age effects on alpha power differ in prefrontal and motor regions. 46 participants were recruited, 16 young participants ranging from 19-29 years of age and 30 older adults ranging from 67-83 years of age. Participants performed a visuomotor precision grip tracking task with a 6-axis-Mini-Model-force transducer (8 trials of 15 seconds each). Tracking variability was operationalized by use of the root mean square error (RMSE). Task-related power (TRPow) in the lower (8-10 Hz) and upper (10-12 Hz) alpha frequency band was calculated for the following electrodes of interest: contralateral motor cortex (C3) and prefrontal electrodes (Fp1, Fp2, AF3, AF4). Older adults showed lower fine motor control performance as compared to young participants. First analyses revealed that in the lower alpha band $TRPow_{C3}$ did not differ between age groups, whereas in analyzed prefrontal electrodes older adults revealed a smaller TrPow decrease than young adults. This smaller decrease mainly resulted from smaller alpha power at rest of the older participants. In the upper alpha band older adults exhibited a significantly smaller $TRPow_{C3}$ than young adults. In contrast to the lower alpha band, no age effect was found in the upper alpha band for frontal electrodes. Task-related power in lower and upper alpha bands seems to reflect different (sensorimotor/cognitive) processes. Our results fit to the findings that the upper alpha frequency band reflects activity in sensorimotor areas (Manganotti et al., 1998), whereas lower alpha might be linked to cognitive performance (Klimesch, 1999). Further, diminished alpha power and TRPow decrease seem to be responsible mechanisms of age-related performance decline. Both cognitive and motor processes seem to be involved in performing the visuomotor tracking task and are both diminished in older adults.
References: Berchicci, M., et al. (2012). Prefrontal hyperactivity in older people during motor planning. *NeuroImage, 62*(3), 1750-1760. Klimesch, W. (1999). EEG alpha and theta oscillations reflect cognitive and memory performance: A review and analysis. *Brain Research Reviews, 29*(2), 169-195. Manganotti, P., et al. (1998). Task-related coherence and task-related spectral power changes during sequential finger movements. *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control, 109*(1), 50-62.

Disclosures: L. Huebner: None. **B. Godde:** None. **C. Voelcker-Rehage:** None.

Poster

078. Motor and Sequence Learning

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 78.06/U39

Topic: F.01. Human Cognition and Behavior

Title: A cognitive framework for explaining serial processing and sequence execution strategies

Authors: *W. B. VERWEY[1,2], C. H. SHEA[2], D. L. WRIGHT[2];
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Abstract: Behavioral research produced many task-specific cognitive models that do not say much about the underlying information processing architecture. Such an architecture is badly needed to understand better how cognitive neuroscience can benefit from existing cognitive models. This problem is especially pertinent in the domain of sequential behavior where behavioral research suggests a diversity of cognitive processes, processing modes and representations. Inspired by decades of reaction time (RT) research with the Additive Factors Method, the Psychological Refractory Period paradigm, and the Discrete Sequence Production task, we propose the Cognitive framework for Sequential Motor Behavior (C-SMB). We argue that C-SMB accounts for cognitive models developed for a range of sequential motor tasks (like those proposed by Keele et al., 2003; Rosenbaum et al., 1983, 1986, 1995; Schmidt, 1975; Sternberg et al., 1978, 1988). C-SMB postulates that sequence execution can be controlled by a central processor using central-symbolic representations, and also by a motor processor using sequence-specific motor representations. On the basis of this framework we present a classification of the sequence execution strategies that helps researchers to understand better the cognitive and neural underpinnings of serial movement behavior.

Disclosures: W.B. Verwey: None. **C.H. Shea:** None. **D.L. Wright:** None.

Poster

078. Motor and Sequence Learning

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: NWO ORA Plus

Title: Age effects on the transfer of sequence knowledge between different types of movements

Authors: *J. S. BARNHOORN[1], F. DÖHRING[2], E. H. F. VAN ASSELDONK[1], W. B. VERWEY[1];
[1]Univ. of Twente, Enschede, Netherlands; [2]Saarland Univ., Saarbrücken, Germany

Abstract: The goal of the current study was to determine whether age influences the ability to transfer sequential knowledge between two different types of motor responses. During practice of a discrete sequence production task, participants responded either by unimanual key presses (KP) on a standard computer keyboard, or by moving a lever with a flexion extension (FE) motion of their right arm. Sequence knowledge was then tested with the other type of responses. Stimulus presentation was identical over the whole task. Sequence learning theory suggests that performers first develop a cognitive representation and with additional practice a motor representation. Based on this insight and on previous research, we hypothesized that elderly would be able to transfer sequence knowledge between the two response modes. Because KP responses are less precise, we expected this condition to be more cognitively controlled than the more difficult FE condition, and therefore also hypothesized more transfer from practice using KP followed by a retention test using FE than vice versa. We tested 32 right-handed elderly (65 - 74) and 27 young people (18 - 30). Individual characteristics were described using the MOCA, a visuospatial working memory task, a digit symbol substitution task and a general health questionnaire. The experiment started with familiarization with KP and FE. Then, two 6-element sequences were practiced with KP or FE for a total of 288 trials. Participants were instructed that two fixed sequences were presented, but not that a test on transfer to another response mode would follow. After an explicit sequence knowledge questionnaire, the test phase with the remaining type of movements followed. The test phase consisted of one block of 24 familiar trials and one block of 24 random trials. Transfer was defined as the percentage speed difference between the familiar and random test blocks. After FE practice, both age groups showed transfer of sequence knowledge. This effect was larger for young participants than for elderly. After KP practice, only young participants showed signs of transfer, which was smaller than the amount of transfer after FE practice. Because there was no difference in the amount of explicit sequence knowledge between the practice conditions, we conclude that there is either more implicit spatial sequence learning in the FE than in the KP response mode, or that KP responses are a more sensitive measure of implicit sequence knowledge. We found that elderly are indeed able to transfer sequence knowledge but to a lesser extend than younger people and that in both age groups response mode plays an important role in measuring such effects.

Disclosures: J.S. Barnhoorn: None. **F. Döhring:** None. **E.H.F. Van Asseldonk:** None. **W.B. Verwey:** None.

Poster

078. Motor and Sequence Learning

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 78.08/U41

Topic: F.01. Human Cognition and Behavior

Support: DFG PA774/12-1

Title: Age related differences in scheduling observational and physical practice

Authors: *F. DÖHRING, S. PANZER;
Human Movement Sci., Saarland Univ., Saarbruecken, Germany

Abstract: Aging is associated with changes in the neuronal structures, which result in cognitive and motor slowing. Observational practice (OP) can be an important tool for motor skill learning especially when the performer has restrictions in executing the task as a result of his physical or cognitive constitution. Thus it might be of particular relevance for elderly. Little attention has been drawn to OP and aging (Celnik et al., 2006). The present experiment was designed to determine whether older (OA) and younger (YA) adults benefit from different OP schedules in learning a sequential motor task. YA (20 to 30 years) and OA (65 to 75 years) had to execute a 10-element movement sequence with their dominant right arm. They used a horizontally mounted lever to control a cursor on a projector screen with motions of flexion and extension. The goal of the task was to move the cursor as quickly and accurately as possible between the illuminated targets. YA and OA were randomly assigned to one of two different practice schedules, a blocked version (BLO) with OP first followed by physical practice (PP) and an alternated version (ALT) with a continuous change between OP and PP. PP consisted of 16 blocks of 10 consecutive sequences for a total of 160 sequences. OP consisted of a video session composed of additional 16 blocks observing a learning model. A 2x2x16 (age-group, schedule, block) ANOVA with repeated measurements for the mean element duration revealed an interaction of Block x Schedule, $F(15,630) = 7.64$, $p < .01$, $\eta^2 = .15$, a main effect in block, $F(16,630) = 93.05$, $p < .01$, $\eta^2 = .69$, and a main effect of age-group, $F(1,42) = 130.44$, $p < .01$, $\eta^2 = .76$. While YA were superior in movement time, both age-groups increased their performance regardless of their schedule. In addition the schedules induce the same pattern without differences in relation to the age-group; subjects in the BLO showed only an advantage in performance compared to subjects in the ALT until bock 4 after which both schedule lead to similar outcome. The difference in performance between YA and OA is consistent with the phenomenon of age-related general slowing. The advantage induced by the BLO can be explained by the greater amount of information provided to the subjects. That YA and OA showed the same pattern of results indicates that both age-groups were able to extract equal information form observing the model, but OA performed slower on the executive level. Further research should address the question whether these effects are more cognitive or motor related.

Celnik, P., Stefan, K., Hummel, F., Duque, J., Classen, J., & Cohen, L. (2006). Encoding a motor memory in the older adult by action observation. Neuroimage. 29(2), 677-684.

Disclosures: F. Döhring: None. **S. Panzer:** None.

Poster

078. Motor and Sequence Learning

Location: Hall A

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Support: German-Israeli Foundation

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Middle East Regional Cooperation Program

Ministry of Science and Technology, Israel

Title: Functional Connectivity patterns in the cerebellar-thalamic-cortical network predicts retention in locomotor adaptation

Authors: *L. SHMUELOF[1], S. BAR-HAIM[2], F. MAWASE[3,4];
[1]Motor Performance Lab, Dept. of Neurol., [2]Physical Therapy, [3]Biomed. Engin., Ben-Gurion Univ. of the Negev, Beer-Sheva, Israel; [4]Dept. of Physical Med. and Rehabil., Johns Hopkins Univ., Baltimore, MD

Abstract: Locomotor adaptation has been studied extensively in recent years as a model for rehabilitation of walking deficits and for motor adaptation. While behavioral results in the lab suggest that locomotor adaptation shares key similarities with reaching adaption, the neural basis of locomotor adaptation is still largely unknown. We investigate the neural basis of locomotor adaptation and retention by examining the relationship between functional connectivity in resting state fMRI activation and the ability to learn and retain novel locomotor patterns. In light of previous results in human patients and nonhuman primates, we focus on the connectivity within the cerebellar-thalamic-cortical pathway, which have shown to be involved in forming and learning motor memories. We were specifically interested in asking whether baseline connectivity levels are indicative of the ability of subjects to learn and retain novel locomotor

patterns and how functional connectivity between regions of interest changes following learning. Thirteen subjects participated in a protocol of three consecutive days. In the first day, subjects did a baseline session in which they were instructed to walk on a split-belt treadmill with no speed perturbations. Subjects were then transported to the MRI scanner and underwent anatomical, functional and structural MRI. In the second day, subjects adapted to the split-belt treadmill with the belts of the treadmill moving at different speeds, and then transported to the scanner and underwent another MRI session. On the third day, in order to evaluate the level of task's retention, subjects performed a behavioral session of adaptation-washout-readaptation protocol. We found that the baseline connectivity between cerebellum and thalamus predicted savings (i.e. rate of relearning) across individuals while baseline connectivity between thalamus and motor cortex predicted the retention (i.e. initial bias of relearning) of the motor task across individuals. These results suggests that the formation of new motor memories is not affected only by the activation of the cerebellar-thalamic-cortical network during the learning, but also by the pattern of connectivity between these areas before learning began. Moreover, we detected a significant change in functional connectivity both between the cerebellum and thalamus and between the cerebellum and motor cortex following motor learning, indicating that the consolidation process is associated with connectivity changes that occur following the learning.

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Poster

078. Motor and Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant AG031769-01

Title: Error estimation training enhances motor learning in older adults

Authors: *Y.-T. CHEN, M. KWON, A. CASAMENTO MORAN, M. W. BEIENE, B. G. GRUBBS, F. T. FIOL, K. GAUGER, E. A. CHRISTOU;
Applied Physiol. and Kinesiology, Univ. of Florida, Gainesville, FL

Abstract: Error estimation, defined as the ability to detect the difference between the desired and actual motor performance, is a crucial part of motor learning. Nonetheless, it is unknown whether training that emphasizes error estimation could improve motor learning in older adults. The purpose of this study, therefore, was to determine whether error estimation training enhances

motor learning in older adults. Twenty seven older adults (71.3 ± 9.1 yrs) participated in this study. They were randomly assigned into three groups (error estimation training group, N=9; typical training group, N=9; and control group, N=9). The error estimation training group practiced 100 trials of a rapid goal-directed task with the ankle joint and estimated their own performance by reporting the endpoint coordinates (space and time) of the peak displacement after each trial. The typical training group practiced the same task for 100 trials but without estimating performance after each trial. The control group did not practice the task with the ankle joint. All the participants were tested one day later with elbow flexion (ipsilateral transfer). The goal of the task was to accurately match a spatial-temporal target. The spatial target was 9º for ankle dorsiflexion and 18º for elbow flexion and the time target was 180 ms. Visual feedback of the position trajectory and target was provided for 5s for all the participants after each trial. We quantified endpoint error as the shortest distance between the peak movement and the target. Participants in the error estimation training group exhibited greater endpoint error than the typical training group (39.8 ± 3.9% V.S. 29.8± 2.6%) during task acquisition (ankle dorsiflexion). In contrast, the error-detection training group (40.6±3.0%) exhibited lower endpoint error than the typical training (55.2.6±7.6%) and control (56.2.3±8.1%) groups during ipsilateral transfer. In summary, these results suggest that error-detection training can enhance motor learning in older adults.

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Poster

078. Motor and Sequence Learning

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Program#/Poster#: 78.11/V2

Topic: F.01. Human Cognition and Behavior

Support: NSF CNS-1228357

Title: Rapid learning of higher-order statistics in implicit sequence learning

Authors: K. R. THOMPSON[1], *P. J. REBER[2];
[1]Psychology, [2]Dept Psychol, Northwestern Univ., Evanston, IL

Abstract: Implicit learning involves extracting experienced regularities and statistical variation from the environment in order to improve behavior. Because knowledge of environmental structure is acquired outside of awareness, it is challenging to determine the precise nature of the

information that is obtained from experience. A commonly used paradigm to study this implicit learning process is perceptual-motor sequence learning in which a covertly embedded sequence is used to create statistical structure across sequences of actions. It is somewhat intuitive to hypothesize that participants acquire the simplest possible statistics, which should be the least computationally demanding to track across time. In most sequence learning experiments, this means learning second-order conditional probabilities (or trigrams). In the current experiments, we manipulated the statistical information available to participants during training on an implicit sequence learning task. Participants were either trained with a traditional 12-item repeating sequence, or with probabilistic, pseudo-randomly mixed 6-item fragments of that sequence constructed to match the lower-order (trigram) statistics of the repeating sequence condition. If only the simplest necessary statistics are learned, we would expect participants to display equal sequence learning across conditions. We found that participants who trained on full repetitions of the sequence exhibited robustly better sequence learning than participants who completed fragment training. This implies that some higher-order statistics are acquired rapidly during repeating sequence practice. We built a computational simulation model to test specific hypotheses about candidate learning processes. The best-fitting statistical learning model incorporated immediate acquisition of fourth-order conditional probabilities, two orders of magnitude more complex than strictly necessary to learn the sequence. A third behavioral experiment confirmed that fragments training led to equivalent learning as repeated sequence training when fourth-order information was matched. This is particularly striking considering the exponential increase in storage capacity necessary to compute higher-order statistics among all elements of the environment. While our results do not rule out other approaches to the computationally difficult problem (e.g., chunking mechanisms), both the behavioral and modeling data suggest that participants rapidly learn higher-order statistics from sequential information.

Disclosures: K.R. Thompson: None. **P.J. Reber:** None.

Poster

078. Motor and Sequence Learning

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 78.12/V3

Topic: F.01. Human Cognition and Behavior

Support: LASPAU

Title: The influence of biomechanics and cognitive demands on locomotor sequence learning

Authors: G. BORIN, *J. T. CHOI;
Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Visual information is naturally used to modify our walking patterns. Voluntary control must be integrated with locomotor pattern generation to control precise stepping movements. Here we challenged walking control by presenting stepping targets of varying spatial complexities (i.e., random vs. ordered), and determined how biomechanical factors (i.e., step length, step width) and cognitive demands (i.e., dual task) affect the rate and magnitude of locomotor sequence learning. Thus far, we tested 8 healthy subjects (27 ± 3 yrs, 2M/6F) walking at 2.0 m/s. The learning paradigm was derived from the serial reaction time task, introduced by Nissen and Bullemer (1987). Performance was measured across 7 blocks that each consisted of 100 steps (Fig. 1a). Blocks 1, 2 and 6 were called random blocks (R1, R2, R3); stepping targets appeared in randomly selected series of location. Blocks 3-5 and 7 were called sequence blocks (S1-S3, S4); subjects were presented with a repeating sequence (e.g., short-long-normal-long-short-normal, narrow-wide-normal-wide-narrow-normal). Sequence-specific learning was calculated as the difference in performance between training block S3 and random block R3; non-specific learning was calculated as the difference between random blocks R2 and R3. We tested two different biomechanics conditions: one required step length modification along the anterior-posterior axis, and the other required step width modification along the mediolateral axis. In addition, a separate group will be tested to determine the effects of a dual cognitive task on the rate and magnitude of locomotor sequence learning (Fig. 1b). The results suggest that both step length and step width sequences could be learned over 300 training steps. Group averaged performance improved over training blocks S1 to S3. The decrease in performance in the last random block R3 indicates sequence-specific effects rather than non-specific learning effects. Moreover, endpoint error in the mediolateral direction was consistently smaller compared to that in the anterior-posterior direction.

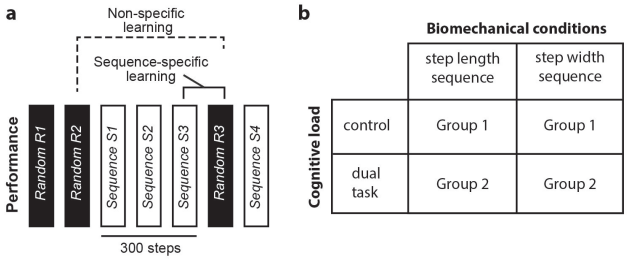

Figure 1. a) Sequence learning paradigm. **b**) Study design and experimental groups.

Disclosures: G. Borin: None. **J.T. Choi:** None.

Poster

078. Motor and Sequence Learning

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 78.13/V4

Topic: F.01. Human Cognition and Behavior

Support: Swedish Scientific Council, Dnr 521-2010-3195

Title: Transfer of sequence-specific and non-specific motor skills after constant and variable training

Authors: *D. M. MUSSGENS[1,2], F. ULLÉN[2];
[1]NINDS, Bethesda, MD; [2]Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: Retention of motor skills is known to be affected by the schedule under which a motor skill is practiced, with variable practice (i.e. varying practice between different tasks) typically being superior to constant practice (i.e. repeating the same task in large blocks). However, it is less clear whether variable practice is similarly beneficial for skill transfer and which components of task performance are transferred between tasks. To determine how practice schedule affects transfer of implicitly learned motor sequence skills we trained two groups of participants on an implicit serial reaction time task and evaluated performance on transfer sequences before and after practice. Participants in the Constant training group practiced 10 blocks of the same 12-element sequence, whereas the Variable group practiced a total of 10 blocks, but alternating between two different 12-element sequences. We evaluated three different types of transfer: task-general transfer to a structurally non-overlapping sequence, inter-manual transfer to a perceptually identical sequence performed with the untrained hand, and sequence-specific transfer to a novel sequence that shared 3 sub-sequences (triplets) with the trained sequences. All sequences were carefully balanced for salient structural properties and transfer was quantified as reaction-time decreases after training as compared to baseline. We found less task-general transfer to the non-overlapping sequence after constant practice than after variable practice, but no difference between groups for inter-manual and sequence-specific transfer. Further, we found evidence for transfer of sequence-specific knowledge in both groups. Transfer was larger to the structurally overlapping sequence than to the non-overlapping sequence and improvements on predictable sequence elements (i.e. the last elements of shared triplets) were greater than those on comparable unpredictable elements (i.e. the first elements of shared triplets). These results suggest that variable practice is more beneficial than constant practice for task-general transfer of sequential skills, possibly by reducing negative interference from implicit sequence expectations. Further, the presence of sequence-specific transfer in both groups suggests that knowledge of sequence structure can, at least partially transfer to novel sequences, independent of practice schedule.

Disclosures: D.M. Mussgens: None. **F. Ullén:** None.

Poster

078. Motor and Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant F31NS087835

Provost Multidisciplinary Award from University of Rochester

Sproull Graduate Fellowship from University of Rochester

Title: Explicit knowledge in a motor sequence depends on strategy

Authors: *M. JAYNES, M. SCHIEBER, J. MINK;
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Abstract: Explicit knowledge of a motor sequence is defined as the ability to describe rules or underlying patterns in the movements that make up the sequence. In experimental paradigms this knowledge may be promoted by specific instruction or developed with practice. Depending on the motor task, explicit knowledge may hinder, benefit, or have no obvious effect on performance. Despite comparable performance improvement, some healthy subjects fail to attain explicit knowledge of underlying patterns in a certain motor sequence. Cognitive and procedural differences between subjects who reach and subjects who do not reach explicit knowledge are unknown. We investigated whether attainment of explicit knowledge in a sequence depends on motor strategy. Healthy adult human subjects performed a battery of finger tapping sequences while wearing a CyberGlove. The tapping tasks included changing visual stimuli that prompted the same finger movements. We instructed subjects to tap the sequences as quickly and accurately as possible but made no insinuation of the common motor sequence. 47% of subjects attained explicit awareness of the underlying pattern in at least one set of motor sequences. A generalized procrustes analysis of the velocity-acceleration plot of each fingertip revealed that subjects who reached explicit knowledge tapped with more consistent finger trajectories throughout the experiment than did subjects who did not reach explicit knowledge. This consistency was present even on sets of motor sequences before the acquisition of explicit knowledge. Despite these differences in the ability to declare underlying patterns in the sequences, there was no difference between groups in performance improvement as measured by tapping speed. Explicit knowledge of an underlying pattern in a finger tapping task is promoted by kinematic consistency, and this kinematic consistency suggests a difference in motor strategy.

This finding suggests that a motor learning strategy resulting in more consistent and automatic movement may be advantageous in motor tasks that benefit from explicit knowledge.

Disclosures: **M. Jaynes:** None. **M. Schieber:** None. **J. Mink:** None.

Poster

078. Motor and Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Support: KTIA NAP 13-2-2015-0002 (DN)

János Bolyai Research Fellowship of the Hungarian Academy of Sciences (KJ)

Title: Long-term stability of implicit sequential memory: One-year consolidation of probabilistic sequence learning

Authors: ***A. KÓBOR**[1], K. JANACSEK[2,3], Á. TAKÁCS[3], D. NEMETH[2,3];
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Abstract: Probabilistic sequence learning (PSL) underlies the efficient processing of statistical patterns in our environment and it is therefore crucial in many day-to-day activities. PSL is acquired gradually; however, only limited information is available about its long-term retention. Former studies emphasized the role of frontostriatal circuits in PSL. The aim of the present study was to investigate the plasticity of these circuits involved in the consolidation and one-year retention of PSL. Healthy young adults (N = 46) performed the Alternating Serial Reaction Time (ASRT) task, which separately measures the sequence-specific and general skill learning component of PSL. The task was administered in three testing sessions: before and after a 24-hour delay and after a one-year delay. We found evidence for improved PSL after 24-hour delay. Specifically, sequence-specific performance was higher at the beginning of the second session than at the beginning of the first session, which also excludes the possibility of simply relearning sequential regularities. Crucially, results showed the one-year retention of this sequence-specific knowledge indicated by similar performance during the second and third sessions. In contrast, general skill learning decreased after one year. The degree of sequence-specific retention for one year was not associated with working memory or frontal lobe functions suggesting the

independence of automatic and controlled adaptive processes. In sum, these results highlight the long-term plasticity of frontostriatal circuits mediating formation, consolidation, and retention of memory traces related to sequential regularities. In addition, findings give insight to the dynamic change of multiple processes that occur during an offline learning period within a longer stretch of time.

Disclosures: **A. Kóbor:** None. **K. Janacsek:** None. **Á. Takács:** None. **D. Nemeth:** None.

Poster

078. Motor and Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant MOP-97830

Title: Changes in NREM2 sleep spindle frequency play a causal role in motor sequence learning consolidation

Authors: ***S. LAVENTURE**[1], S. FOGEL[2], G. ALBOUY[3], O. LUNGU[1], C. VIEN[1], P. SÉVIGNY-DUPONT[1], C. SAYOUR[1], J. CARRIER[1], H. BENALI[4], J. DOYON[1];
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Abstract: Motor sequence learning refers to the process by which simple, stereotyped movement elements come to be performed effortlessly as a unitary sequence through multiple sessions of practice. Past studies have shown that motor sequence learning goes through different phases during practice, hence allowing the memory trace to become more stable over time, a process called motor memory consolidation. Numerous researches have convincingly demonstrated that sleep plays a critical role in motor sequence learning consolidation. Yet there is no consensus regarding the sleep stages implicated in this type of memory consolidation. Evidences indicate that NREM2 sleep, and spindle activity in particular, are critical for motor memory consolidation to occur but most of these studies are only correlational in nature. To probe the possible causal role of NREM2, we implemented a targeted memory reactivation (TMR) protocol. We conditioned a first group of participants (n = 25) with a rose-like odor during learning of a sequence of finger movements, and re-exposed them to the odor during subsequent NREM2 sleep (Cond-NREM2). A second group (n = 23) was conditioned with the same odor and was re-

exposed during REM sleep (Cond-REM). Finally, a third group (n = 28) was not conditioned, but was exposed to the olfactory stimulus during NREM2 sleep (NoCond). In order to measure the effect of cuing on consolidation of the novel task we retested all subjects in the morning. We found significant difference of gains in performance between the experimental groups ($F2, 71 = 5.855$, $p = .004$). More precisely, the Cond-NREM2 group had significantly higher gains in performance than both the Cond-REM and NoCond groups. Importantly, Cond-NREM2 showed significant increases in sleep spindles of several characteristics over parietal regions when comparing periods of sleep before and during stimulation. More importantly, however, we found that the change in frequency of sleep spindles at Pz during stimulation was specific to the 13.5 to 14 Hz frequency band, and mediated the relationship between our experimental groups and the offline gains. These findings strongly suggest that NREM2 sleep is causally implicated, through the activity of sleep spindles, in the consolidation of motor sequence memories.

Disclosures: **S. Laventure:** None. **S. Fogel:** None. **G. Albouy:** None. **O. Lungu:** None. **C. Vien:** None. **P. Sévigny-Dupont:** None. **C. Sayour:** None. **J. Carrier:** None. **H. Benali:** None. **J. Doyon:** None.

Poster

078. Motor and Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Support: PIEF-GA-2013-62772

Title: Predicting individual differences in sequence learning from oscillatory activity in human MEG-data

Authors: ***F. ROUX**[1], R. FROST[2,3], M. CARREIRAS[4,5,6];
[1]Basque Ctr. On Cognition, Brain and Language, Donostia/San Sebastian, Spain; [2]Dept. of Psychology,, The Hebrew Univ. Jerusalem, Jerusalem, Israel; [3]BCBL. Basque Ctr. on Cognition, Brain and Language, San Sebastian, Spain; [4]BCBL. Basque Ctr. on Cognition, Brain and Language., San Sebastian, Spain; [5]Ikerbasque, Basque Fndn. for Sci., Bilbao, Spain; [6]UPV/EHU, Univ. del Pais Vasco, Bilbao, Spain

Abstract: Many complex behaviours involve the ability to learn and reproduce sequences of events in their correct temporal order. Some of these sequences can occur embedded in continuous streams of information. Therefore, we often extract sequences from background

information without any prior knowledge about the timing of sequence on- and offsets. While recent theoretical and empirical work indicate that the sequential structure of inputs may be inferred from perceived transitional probabilities, the ability to exploit such statistical information seems to be highly variable. A mechanistic account of what is causing such differences in behaviour, however, is still missing. Here we address how the brain extracts the statistical regularities embedded in temporal sequences and whether inter-individual differences in sequence learning can be predicted from fluctuations of brain activity. Building on previous electrophysiological studies, we hypothesised that rhythmic brain activity supports the representation of behaviourally relevant sequences in neuronal activity as well as their consolidation in memory. Our findings suggest that transitional probabilities embedded in temporal sequences are tracked by oscillatory activity in multiple frequency bands (1-50Hz). Finally, we observed that individual differences in sequence learning can be predicted with high accuracy from delta (1-4Hz) coupled beta (15-20Hz) oscillations.

Disclosures: **F. Roux:** None. **R. Frost:** None. **M. Carreiras:** None.

Poster

078. Motor and Sequence Learning

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Program#/Poster#: 78.18/V9

Topic: F.01. Human Cognition and Behavior

Title: The impact of predictability on implicit motor and perceptual sequence learning

Authors: L. KATZ, B. FLYNN, C. SINGH, C. SEMERJIAN, L. IZRAYLOV, M. MALABANAN, J. CUDIA, *L. H. LU;
Psychology, Roosevelt Univ., Chicago, IL

Abstract: This study aimed to evaluate if the rate of implicit sequence learning differs across motor and perceptual modalities, and how the predictability of a sequence affects learning. We used a serial response time (SRT) paradigm and assessed learning via a decrease in reaction time (RT). Motor and perceptual modalities were decoupled: participants “caught” an auditory cue (A, B, C, or D) by pressing the key corresponding to one of the four locations on the screen that matched the auditory target. In the motor condition, the motor response to “catch” the target location repeated in a 7-digit sequence. In the perceptual condition, the auditory cues repeated in a 7-digit sequence, but there was no sequence to motor responses corresponding to auditory targets. Sequences were primarily first order conditional (FOC), where knowing one digit sufficiently predicts the next sequence, or second order conditional (SOC), where two digits are

needed to predict the next in the sequence. The first four blocks were learning blocks, the fifth block was composed of unpredictable trials (random), and the sixth block was identical to the learning blocks. Right-handed healthy adults participated (n=40; 37 females; age=23.67, sd=6.40). Across blocks 1-4, we evaluated for learning by a within-subject 2 (modality) x 2 (predictability) x 4 (blocks) ANOVA. We found main effects for predictability ($F(1,39)= 14.95$, $p < .01$), block ($F(3,117)= 11.17$, $p < .01$), and modality by predictability interaction ($F(1,39)= 4.88$, $p = .03$). Participants responded faster to the FOC sequence and across blocks. The FOC advantage was only observed in the motor sequence. These findings indicate that learning occurred regardless of sequence modality, though FOC yielded faster RTs when learning a motor sequence. We also examined the stability of learning by comparing RT to the learned sequence after a block of random trials. This within-subject 2 (modality) x 2 (predictability) x 2 (block 5 vs. 6) ANOVA yielded main effects for predictability ($F(1,39)= 14.99$, $p < .01$), block ($F(1,39)= 26.01$, $p < .01$), and predictability by block interaction ($F(1,39)= 12.07$, $p < .01$). Participants responded faster to the FOC sequence and to block 6. The interaction showed that RT decreased more for the FOC sequence than for the SOC sequence. Altogether, these results suggest that sequence predictability affects implicit sequence learning, but there was no evidence to support that motor and perceptual sequences have different rates of learning.

Disclosures: **L. Katz:** None. **B. Flynn:** None. **C. Singh:** None. **C. Semerjian:** None. **L. Izraylov:** None. **M. Malabanan:** None. **J. Cudia:** None. **L.H. Lu:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

Location: Hall A

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Program#/Poster#: 79.01/V10

Topic: F.01. Human Cognition and Behavior

Support: CNC.IBILI-UID/NEU/04539/2013

Title: Fluctuations in processing time are reflected in the shape of early visual evoked potentials

Authors: ***M. J. RIBEIRO**[1], J. S. PAIVA[2], M. CASTELO-BRANCO[2];
[1]Univ. Coimbra, Coimbra, Portugal; [2]IBILI, Fac. of Med., Univ. of Coimbra, Coimbra, Portugal

Abstract: Fluctuations in sustained attention lead to moment-to-moment fluctuations in task performance. Even in easy repetitive visuomotor tasks, human performance fluctuates with time. These variations in performance have been linked to fluctuations in the activity of the attention networks. Attention has an impact in early stimulus processing, yet, the relationship between

moment-to-moment fluctuations in task performance and early stimulus processing is still not clear. In this study, we explored this relationship by taking advantage of the high temporal resolution of EEG signals, and adopting a within-subject, whole time course analysis of single trial ERP data. We studied the neuronal correlates of reaction time variability in a simple visuomotor two-choice reaction time task. The task was chosen in order to minimize speed-accuracy tradeoffs while maintaining the requirement for overt attention on the visual stimulus. We acquired EEG recordings during task performance, from healthy young adults, using a 64-channel Neuroscan system. In order to determine if trial-to-trial differences in visual evoked responses might be caused by slight shifts in the position of gaze fixation at the time of stimulus onset, we also recorded gaze position during task performance with a dark pupil eye tracking system, iView X (SensoMotoric Intruments - SMI). Our results revealed that visual evoked potentials in a time window between 150 and 300 ms after stimulus onset correlate with reaction time on a trial-by-trial basis. Within this time window, the visual evoked potentials presented a marked negative peak with bilateral occipito-parietal topography (N1/N170). Trials associated with faster responses, presented a sharper N1 potential, with a narrower distribution in time and higher peak amplitude, while slower trials were associated with smaller amplitudes and a broadening of the N1 shape in time. Eye gaze position at time of stimulus onset did not correlate with these ERP amplitude values, suggesting that these differences were not related to differences in external input but, most likely, were related to moment-to-moment differences in internal state. In conclusion, our observations suggest that fluctuations in reaction time reflect trial-to-trial differences in the amplitude of visual evoked potentials as early as 150 ms after stimulus onset. The time course and topography of these neural responses suggest that these originate in the ventral extrastriate cortex, a visual area sensitive to feature-based attention.

Disclosures: **M.J. Ribeiro:** None. **J.S. Paiva:** None. **M. Castelo-Branco:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

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John Templeton Foundation Grant #48192

Title: Individual differences in steady-state visual evoked potential measures of attention predict psychological differences during decision making

Authors: *M. D. NUNEZ[1], J. VANDEKERCKHOVE[2], R. SRINIVASAN[2];
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Abstract: Visual attention allows individuals to preferentially process relevant stimuli and suppress processing of irrelevant stimuli during perceptual decision making tasks. Steady-state visual evoked potentials (SSVEPs) are defined as narrow band EEG responses at flicker frequencies of visual stimuli. Steady-state responses have been previously shown to reflect differing attentional abilities between subjects. In this study we were able to predict individual differences in specific psychological processes during visual decisions by comparing subjects' SSVEP responses, a proxy for visual attention. Subjects were asked to perform a novel two-alternative forced choice task as accurately as possible within a time constraint. On each trial subjects decided whether a Gabor was drawn from a distribution of high or low spatial frequency and indicated their choice using a response box. SSVEPs were evoked during the experiment at both 20 and 30 Hz in the EEG spectrum, corresponding to the flicker frequency of the Gabor and the visual noise respectively. Individual differences in accuracy and reaction time are shown to be predicted by individual differences in the phase information of the SSVEPs, specifically the phase-locking index (PLI) across trials at 20 and 30 Hz, especially in electrodes over the parietal and occipital lobes. This predictive ability is thought to be due to differences in individuals' ability to suppress visual noise and enhance visual signal. Furthermore these individual differences in attention ability predict specific psychological parameters of the diffusion model, a model of speeded decisions that assumes specific psychological processes on each trial. By modeling reaction time and choice as a diffusion process, we reparameterized responses into a distribution with parameters that provide the average evidence accumulation rate, variance in evidence accumulation, non-decision time, and decision criterion per trial. Subjects who suppressed visual noise well (i.e. had small 30 Hz PLIs) had faster within-trial evidence accumulation rates as well as faster visual preprocessing times. While subjects who enhanced the visual signal (i.e. had large 20 Hz PLIs) had larger within-trial evidence accumulation variance and faster preprocessing times. The accuracy and reaction time of out-of-sample subjects was well predicted given their attentional response to the noise and signal stimuli, as measured by SSVEPs, when using estimated parameters of the diffusion model. Our modeling paradigm with the use of simple EEG measures has the potential to identify individuals that will be best at specific tasks that require visual attention.

Disclosures: **M.D. Nunez:** None. **J. Vandekerckhove:** None. **R. Srinivasan:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

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Topic: F.01. Human Cognition and Behavior

Title: Individual differences in ERP components associated with signal detection and distractor resistance

Authors: H. WILLIAMS[1], K. KIM[4], W. J. GEHRING[2], *C. LUSTIG[3];
[1]Psychology, [3]Dept. of Psychology, [2]Univ. of Michigan, Ann Arbor, MI; [4]Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Responding to the world around us involves detecting important signals, often in the face of distraction. Processing sensory stimulation, deciding whether to attend to it, and responding all result in changes in brain activity that can be using event-related potentials (ERPs). We examined ERP components in 29 healthy young adults while they tried to detect a small, briefly presented signal against either a stable background or a moving background that created distraction resulting in slower and less accurate responses. Approximately 40% of subjects failed to show the N200 component, often associated with early stages of signal detection, in either condition. Behaviorally, these participants had lower scores on measures of signal sensitivity and processing efficiency. In contrast, the P300 component, typically associated with later processing stages including decisions about stimuli (e.g., “is this a signal I should respond to?”) was robust across subjects and sensitive to the distractor manipulation (smaller amplitude, longer latency). Correlational analyses indicated that greater P300 amplitude and shorter latency were associated with better signal sensitivity and processing efficiency. Together the results suggest that early perceptual-attention processes may be important for overall signal detection sensitivity, whereas later attentional components more associated with cognitive control play a role in resisting distraction.

Disclosures: **H. Williams:** None. **K. Kim:** None. **W.J. Gehring:** None. **C. Lustig:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

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Topic: F.01. Human Cognition and Behavior

Support: Medical Research Council

Title: Distinct mechanisms for distractor suppression and target facilitation

Authors: *M. P. NOONAN[1], N. ADAMYAN[3], A. PIKE[2], F. PRINTZLAU[2], B. CRITTENDEN[2], M. G. STOKES[2];
[2]Dept. of Psychiatry, Oxford Ctr. for Human Brain Activity, [1]Univ. of Oxford, Oxford, United Kingdom; [3]Lab. Psychologie de la Perception, Univ. Paris Descartes, Paris, France

Abstract: It is well established that preparatory attention improves processing of task-relevant stimuli (Desimone and Duncan, 1995). Although it is often more important to ignore task-irrelevant stimuli, comparatively little is known about preparatory attentional mechanisms for the inhibition of expected distractions (Ruff and Driver, 2006, Navalpakkam and Itti, 2007). Here, we first establish behaviourally that distractor inhibition is not under the same top-down control as target facilitation. Using a four-location variant of the Posner paradigm, participants were cued to either the location of a target stimulus, the location of a distractor or were provided no predictive information. In addition to varying the presence of the distractor, we critically manipulated the flexibility of cueing: spatial cues were randomised on each trial in one version of the task (Flexible), or fixed for a block of trials (Blocked). In Experiment 1, we found that participants (n=24) were able to use target-relevant cues to facilitate target processing in both blocked and flexible conditions, but distractor cueing was only effective in the blocked version of the task. In Experiment 2, we replicate these findings in a larger sample of participants (n=71), and leveraged the additional statistical power to perform individual differences analyses to tease apart potential underlying mechanisms. We found no evidence for a correlation between these two types of benefit, suggesting that flexible target cueing and distractor suppression depend on distinct cognitive mechanisms. In Experiment 3, we use EEG (n=20) to show that preparatory distractor suppression is associated with a diminished P1 and altered N2pc components, but we found no evidence to suggest that this effect was mediated by top-down control of oscillatory activity in the alpha band (8-12Hz). We conclude that flexible top-down mechanisms of cognitive control are specialised for target-related attention, whereas distractor suppression only emerges when the predictive information can be inferred directly from past experience. This is consistent with a predictive coding model of expectation suppression that is selective for task-irrelevant information. Desimone R, Duncan J (1995) Neural mechanisms of selective visual attention. Annual review of neuroscience 18:193-222. Navalpakkam V, Itti L (2007) Search goal tunes visual features optimally. Neuron 53:605-617. Ruff CC, Driver J (2006) Attentional preparation for a lateralized visual distractor: behavioral and fMRI evidence. Journal of cognitive neuroscience 18:522-538.

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Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

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Support: DFG Grant SFB/TRR 135, TP B3

DFG Grant SCHU 1330/5-1

Title: Can top-down control override learning experience?

Authors: *A. SCHUBÖ, H. KADEL, T. FELDMANN-WÜSTEFELD;
Philipps Univ., Marburg, Germany

Abstract: Recent findings have challenged the classic dichotomy of bottom-up vs. top-down as control mechanisms in the guidance of visual attention and have suggested past selection history, e.g. evident in learning experience, as a third source of selection bias (Awh, Belopolsky, & Theeuwes, 2012). The present study has used event-related potentials to examine how learning experience affects the deployment of visual selective attention, in addition to, and in interaction with top-down control. Learning experience was differentially induced by having participants perform a visual categorization learning task which was combined with a visual search task in the course of the experiment. One group of participants had to respond to color in categorization learning; a second group had to respond to shape. In the search task, all participants had to report the orientation of a line embedded in a shape singleton that served as target. In some trials, an additional color singleton distractor was presented. The crucial manipulation involved the presentation of a cue at the beginning of a trial. The cue indicated what type of task had to be performed so that participants could prepare for the task in an upcoming trial before stimulus onset. The main questions to be investigated were whether learning affected performance in the search task and whether resulting differences in performance could be overcome when the cue allowed task preparation. Results showed that color distractors slowed down performance in the visual search task to a greater extent when color rather than shape was determined as being predictive in the learning task. Disentangling the mechanisms underlying this behavioral effect by means of lateralized ERP components showed differential attention deployment: ERPs in the color group indicated that color distractors captured attention in the search task (as indicated by the ND component) before they could be actively suppressed (as indicated by the PD component). ERP results in the shape group indicated that color distractors did not capture attention (no ND) and that suppression was possible earlier in time (early PD). Interestingly, the findings were virtually the same when a cue indicated the task at the beginning of each trial and allowed participants to prepare for the upcoming task. These results provide direct evidence of

preferential attention deployment towards stimuli with high predictive value in learning, even if these are irrelevant for the search task and participants had the opportunity to actively prepare for the search task in advance.

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Poster

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Support: NIH/NEI 2T32EY015387

Sigma Xi GIAR

NIH/NEI 12576

Title: ERP evidence of reafferent priming of V1 feedforward circuits by spatial attention during dynamic vision

Authors: ***A. ROYSTON**[1], J. NAPAN[1], K. ANDERSON[1], A. HABERMAN[1], S. J. LUCK[1], S. A. HILLYARD[2], W. M. USREY[1], G. R. MANGUN[1];
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Abstract: Efficient selection of task-relevant information and suppression of irrelevant information is crucial for successful goal-directed behavior in everyday life. Classic visual selective attention paradigms in humans often employ briefly flashed stimuli (e.g., Posner Cueing, RSVP), and have shown modulations of visual evoked activity with attention. However, these designs fail to capture the dynamics and complexity of continuously-visible real world visual scenes, leaving open questions concerning the application of these findings to more natural viewing scenarios, perhaps failing to reveal key mechanisms. For example, although electrophysiological studies in nonhuman primates and fMRI studies in humans have reported clear, replicable effects of spatial selective attention in striate cortex and LGN, ERP studies of this issue in humans have yielded inconsistent results. We hypothesize that in natural vision, the presence of continuous inputs may trigger an attention-related reafferent priming of feedforward circuitry in V1 such that subsequent feedforward inputs benefit from enhanced attentional modulation at this early cortical locus. To test this, we presented continuous bilateral stimuli to human subjects who covertly attended to one hemifield in order to detect occasional targets

(contrast decrements) in dynamic and static stimulus conditions. Our preliminary findings using these designs provide support for our hypothesis, showing a significant modulation of ERPs generated in V1 (i.e., the C1 component of the visual ERP) in the dynamic, ongoing stimulus conditions compared with the static stimulus conditions. Our findings suggest that reafferent priming enhances processing of feedforward inputs in human V1 during selective attention to ongoing, dynamic stimulation, which is more similar to the conditions faced in natural vision.

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Poster

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Title: Learning-dependent changes in neural mechanisms underlying selective attention

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Abstract: Competing theories have been proposed to describe neural mechanisms underlying selective attention. These include sensory gain, noise modulation, and efficient read-out mechanisms. While these mechanisms are not mutually exclusive, different studies have demonstrated that one mechanism may outperform another in terms of predicting attention-induced changes in behavioral performance. We hypothesized that these discrepancies may arise in part from changes in attentional modulations during learning. To test this hypothesis, we simultaneously tracked changes in psychophysical contrast thresholds and neural responses using electroencephalography (EEG) while human subjects performed a contrast discrimination task over one month. During the early stages of learning, focused attention increased the multiplicative response gain of the early sensory P1 component as well as the decision-related late positive deflection (LPD or P3). Quantitative modeling reveals during this early phase, gain amplification of the P1 and LPD sufficiently accounted for attention-related improvement in

behavioral performance. However, at later learning stages (after ~17 days), there was no attentional gain modulation in the P1 component. Quantitative modeling revealed that noise-reduction models best accounted for the link between P1 responses and behavior. However, there was sufficient gain amplification of the LPD component that predicted attention-related improvements in behavioral performance. Collectively, these results suggest that neural mechanisms underlying selective attention are stage-dependent: selective attention increases gain amplification of early sensory signals only during initial phases of learning, whereas modulations of the LPD are observed throughout learning stages.

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DAI-ED SHS

Title: Predictability or relevance for the task... Who drives spatial attention? An EEG study

Authors: ***N. DO CARMO BLANCO**[1], J. JOZEFOWIEZ[1], J. J. B. ALLEN[2];
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Abstract: Being able to detect contingencies between events is essential for a wide range of cognitive functions, from motor control to social interaction. This learning process, widely accepted to be prediction driven, could happen even without awareness, when it is not relevant to the task at hand. Prediction is also at the core of the efficient processing of statistical regularities described by predictive coding theories, where neural signals are related to the extent to which stimuli are predicted. This framework is supported by studies showing increased BOLD activity for unpredictable outcomes. Nevertheless, those results could reflect an enhancement of attention when a stimulus is not expected. Here we used the event-related potential N2pc component, widely recognized to reflect allocation of spatial attention, to study how outcome predictability affects attention. If contingencies are learned even when they are task-irrelevant, could such implicit associations also modulate attention? We used an associative learning paradigm where

we manipulated contingency (predictiveness) and relevance of the association upon streams of cue-outcome visual stimuli, while stimulus characteristics and probability were held constant. At the end of each stream, participants had to assess the association between one of the two possible cues and one of the two possible outcomes. To elicit the N2pc component, participants had to indicate the orientation of a random feature of every outcome, which was embedded among distractors. We found that outcomes predicted by a cue showed an increased spatial attention as indexed by the N2pc component, in accordance with a predictive coding model. Surprisingly, this effect was found even when this association was irrelevant. These results show that spatial attention is enhanced by predictability during learning, even when the association is irrelevant to the main goals of the task at hand. Moreover, it indicates that non-contingent outcomes capture spatial attention less efficiently, which suggests that the enhanced BOLD activity found for unpredictable outcomes is not related to attention, supporting a predictive coding framework. The current study confirms the remarkable ability of the brain to extract and update predictive information and the modulation of encoding by top-down processes.

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MRC

Title: Mnemonic target representation in a visual detection task

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Abstract: Many perceptual decisions require the current input to be matched up with an internal decision criterion, or template. Classic studies of visual attention have typically attributed its representation either to raised baseline activity in sensory neurons tuned to the template's features, or to changes in synaptic gain of those neurons. More recent studies, however,

complicate this explanation: the encoding of stimulus features, either in visual and prefrontal neural populations, or in activation patterns distributed across the cortex, changes rapidly from moment to moment, tracing a complex but stereotyped trajectory through activation state space. This prompts a re-evaluation of when in time, and in what representational format, stimulus and template codes are compared. We measured magnetoencephalography as human participants performed a match-to-template orientation task, and measured the temporal dynamics of stimulus representation, template representation, and how both representations interact at the decision stage. Our findings run counter to some classic findings in visual attention, since we did not see a tonic activation of the template, and saw only short-lived cross-generalization in the coding between stimuli and templates when using the same coding space. Instead, template information transiently emerged around the time of stimulus onset, and quickly returned back to baseline, in keeping with an account of template encoding in synaptic weight changes that do not require persistent activity. In addition, we found that the trial-to-trial match between template and stimulus (i.e., the decision variable) was represented in the MEG signal, and that it emerged around the time that template coding began to subside. Intriguingly, we found that the task-irrelevant signed difference between template and stimulus was also encoded at this stage. We argue that this task-irrelevant representation can be explained readily by a probabilistic population code underlying the decision stage. We conclude that a simple, multi-layered architecture based on population codes can provide a framework for bringing together findings across visual attention, working memory, and decision-making.

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Title: How does spontaneous brain activity interact with evoked activity? Non-additive interaction, phase-dependence and scale-free properties

Authors: *Z. HUANG[1], J. ZHANG[2], A. LONGTIN[1], G. DUMONT[1], N. W. DUNCAN[1], J. POKORNY[3], P. QIN[1], R. DAI[2], F. FERRI[1], X. WENG[2], G. NORTHOFF[1];
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Abstract: Investigations on both cellular and regional levels of neural activity showed trial-to-trial variability reduction after stimulus-onset, highlighting the central role of spontaneous brain activity and its ongoing nature during task-evoked activity. However, evidence is still lacking on elucidating the exact relationship between spontaneous and task-evoked activity. On the other hand, to understand this interaction, one may have to trace back to the unique temporal structure of spontaneous activity. Being ubiquitous in nature, scale-free properties (power-law distribution) has been observed in neural activity across many different spatiotemporal scales including the infraslow frequency fluctuations (<0.5 Hz) of spontaneous activity. This property is also characterized as long-range temporal correlations (LRTCs). However, the functional significance of LRTCs and their relationship to spontaneous and task-evoked activity remain unknown. The aim of our study was to use functional magnetic resonance imaging (fMRI) to investigate how spontaneous brain activity interacts with task-evoked activity, as well as how the LRTCs of spontaneous activity relate to this interaction. Using an extremely sparse event-related design, a novel correction approach (accounting for spontaneous fluctuations by pseudo-trials) and phase analysis of fMRI signals, we demonstrate that (i) stimuli presented at a lower magnitude of preceding spontaneous BOLD activity produce a stronger response than stimuli presented at a higher magnitude, which explains trial-to-trial variability reduction after stimulus-onset; (ii) this non-additive interaction was further characterized by phase-dependent excitability of spontaneous activity, i.e. different temporal excitability for trough vs. peak and fall vs. rise phases; (iii) the degree of the phase-dependent excitability is related to the degree of LRTCs of spontaneous activity, as indexed by the power-law exponent. This suggests that the temporal structure of spontaneous activity shapes the non-additive interaction between spontaneous and task-evoked activity. To the best of our knowledge, we have provided, for the first time, direct evidence for a non-additive interaction between spontaneous and task-evoked activity and its underlying mechanism of phase-dependent excitability, as well as its relationship to the temporal structure of the spontaneous activity. Our findings not only contribute to our understanding of spontaneous brain activity and its scale-free properties, but also bear important implications for neural activity in general.

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Title: Task-independent correlations predict task-evoked BOLD response similarity in frontoparietal association cortex

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Abstract: Anatomical and functional connectivity measurements suggest that primate association cortex is organized into distributed large-scale networks. In humans, this has been most effectively demonstrated through analyses of task-independent low-frequency fluctuations in the BOLD signal. Multiple approaches for extracting network structure from these measurements, including clustering, community detection, and independent components analysis, have revealed network definitions that are largely convergent. Moreover, patterns of results in the task-based literature often bear a striking resemblance to these networks. Despite such observations, the precise relationship between task-independent and task-evoked signals remains a controversial issue. To address it, we analyzed fMRI data that were collected while humans performed a demanding perceptual discrimination task with a fast event-related design. We estimated task-evoked responses across different parcellations of frontoparietal association cortex by fitting finite impulse response models to BOLD timeseries data. This produced, for each region in a given parcellation, an evoked response waveform and a timeseries with residual, task-independent variance. We then estimated correlation coefficients within pairs of response waveforms and pairs of residual timeseries. Results showed that estimates of task-evoked similarity and task-independent connectivity were strongly related. Moreover, while response waveforms in much of association cortex were highly reproducible across splits of the data, the responses in some regions markedly differed from canonical models of the hemodynamic response function. Despite being distributed throughout cortex, regions with similar non-canonical responses showed high levels of task-independent connectivity. The strength of the relationship between task-independnt and task-evoked measures also depended on the

parcellation scheme used to define regions: the relationship was stronger when regions were defined using population atlases of task-independent networks than when defining regions with common anatomical landmarks. Together, these results indicate that task-independent connectivity is meaningfully related to task-evoked processing and provide support for efforts to understand cortical organization through the lens of large-scale task-independent networks.

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Poster

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Title: Stimuli-based phasic components of task-induced deactivation during sustained attention

Authors: ***K. SHATTUCK**, J. W. VANMETER;
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Abstract: Background: Task-induced deactivations (TIDs) observed in functional magnetic resonanace imaging (fMRI) studies are recognized as an important component of brain activity during externally focused cognition. In particular, the magnitude of TIDs in the ventromedial prefrontal cortex (vmPFC-TIDs) increases with attentional effort, working memory, and episodic memory formation. Clinical implications include decreased vmPFC-TIDs correlating with symptom severity in depression and schizophrenia. Though TIDs are commonly considered the result of top-down attentional control, there has been a lack of investigation into stimuli-driven, bottom-up TID responses. Using a hybrid block and event-related task design, we investigated both the task-based tonic component and stimuli-based phasic component of vmPFC-TIDs during the Sustained Attention Task (SAT) at two levels of difficulty. Methods: Ten (7 female, 18-39 years) healthy, right-handed subjects with normal or corrected-to-normal vision performed 84-second blocks of the SAT and a version of the SAT with distraction-induced challenge (dSAT) interpersed with 30-second periods of rest while undergoing fMRI scanning. Both the SAT and dSAT briefly presented (40-75 ms) a small visual target on 50% of randomly selected trials (6-8s ISI), and subjects pressed one of two buttons to indicate target detection; the dSAT added flashing background illumination (10 Hz). Results: Group results showed vmPFC-TIDs

from rest that were greater for dSAT than SAT blocks. The group deactivation from rest provided a region-of-interest for a within-task event-related analysis that showed stimuli-related deactivations were greater for target trials than non-target trials. Conclusions: Phasic, stimuli-driven TIDs should be considered as a component of cognitive responses to external stimuli and may provide additional information to our understanding of attention, memory, and disease.

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Title: Intracranial Gamma-band coherence is influenced by task and experience

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Abstract: Cortical gamma band activity (above 30Hz) has been hypothesized to be related to attention allocation, conscious perception, and to the integration of information into a unified representation. A possible mechanism by which local gamma activity can support coordination between remote cortical sites is synchronization. By synchronizing or desynchronizing gamma activation between sites the brain can flexibly create and adjust functional networks to account for changing demands. The fast high-gamma frequency band (65-115 hz) may be especially useful for quickly recruiting and maintaining multiple concurrent networks. To test the hypothesis that high-gamma coherence is adaptively modulated during task performance we computed a network level coherence change from ECoG electrodes implanted in 4 epileptic patients while they performed a task designed to establish audio-visual associations. During each trial subjects categorized a visual stimuli as a body or a tool. Each of these categories was uniquely preceded by auditory stimuli from a specific category (bird songs or musical tunes). A third neutral auditory stimulus was followed by either visual category and was used to emphasize the regularity of the other two auditory categories. Each subject completed multiple task runs (6-8 runs). An additional resting state signal was recorded in a separate run. To evaluate whether

gamma synchronization was modulated by entering a task state and was further shaped by our patients' experience with the task and the novel associations it promoted, we performed two comparisons of pair-wise LFP high-gamma band correlation across all electrodes in each patient: 1) coherence during task vs. coherence during rest; and, 2) and coherence during the first task run vs. coherence during the last task run. Our results show that in both comparisons a larger than chance number of pairs exhibited a coherence change, indicating that gamma coherence is indeed modulated by task and experience. Furthermore, similar electrodes were the most modulated in both comparisons, suggesting that the network that was initially created to perform the task was being actively modulated with experience. These results support the hypothesis that high-gamma band coherence might be a mechanism by which the brain coordinates the integration of information between different cortical sites to support efficient cognitive functioning.

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Title: Gamma-band synchrony measures indicate differential prefrontal and parietal contributions to signal detection and top-down control

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Abstract: Correct detection of environmental cues and resisting distraction are important for successful behavior. These abilities are supported by frontoparietal networks in both humans and rodents, with rodent studies further indicating the crucial role of cholinergic innervation. Gamma-band synchrony may provide an important bridge between the human animal findings, as human encephalogram (EEG) studies link increased gamma-band synchrony to the hemodynamic changes underlying BOLD fMRI, rodent local field potential studies show its modulation by acetylcholine (ACh), and findings from both species indicate its association with both bottom-up and top-down attention. We therefore examined changes in gamma

synchronization in healthy young adults performing a modified version of the distractor condition sustained attention task (dSAT) developed for cross-species studies of signal detection and distractor resistance (Demeter et al., 2008). The major modification was the use of a shifting-grid distractor that more clearly provided a competitive input for attention, as opposed to whole-field changes in luminance that may also challenge lower-level perceptual processes. As expected gamma synchrony increased during task performance and was further increased during the distractor. However, these increases were primarily in left parietal regions rather than the right prefrontal regions predicted from rodent studies. Instead, right prefrontal gamma dispersion was associated with increased response-time variability during distraction, suggesting that this region contributes to signaling the need for top-down control rather than its implementation. Occipital regions showed significant distractor-entrained oscillation patterns, with greater entrainment during "miss" trials, indicating attention misdirected to the distractor. Correlational analyses suggested modulation of distractor entrainment by left-parietal gamma, further implicating parietal cortex as an important component in top-down control. In summary, the present findings are consistent with previous human and rodent studies implicating frontoparietal networks in attention and distractor resistance, but suggest that right prefrontal cortex may be more involved in signaling a need for control, and parietal regions with its implementation.

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Title: Feature-based attention modulates correlated BOLD activity in the visual cortex

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Abstract: An important neural mechanism underlying top-down selective attention is the modulation of synchronous neural activity across brain areas. Attention-induced modulations of interareal synchrony within the visual cortex have been suggested to promote effective transmission of goal-relevant information across neuronal assemblies representing such information. While most prior literature examined attention-related changes in interareal connectivity under spatial attention, it is less understood how feature-based attention modulates activity along specific visual pathways representing the attended features. Here we addressed this issue via functional magnetic resonance imaging when human participants performed a cued-attention task during which an auditory cue instructed them to attend covertly to either the direction of motion or color of a moving-dot stimulus. We found that compared with a passive-viewing condition, blood-oxygen-level-dependent (BOLD) activities within the extrastriate areas, including V3, V3A, hV4, and MT+, were enhanced following an attentional cue. Preliminary analyses of interareal correlations in cue-induced BOLD activities reveal that attention modulated the overall correlation of trial-to-trial fluctuations in BOLD responses across the visual areas. Interestingly, reductions in correlated BOLD activities were observed among areas along the dorsal visual stream when participants attended to the color instead of the direction of the dots. These results are consistent with the view that feature-based attention enhances the representation of goal-relevant sensory information by modulating connectivity both among the entire visual system and along specific visual pathways.

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Title: Causal role of IPS and TPJ in selective attention in multi-target environments

Authors: *M. PRAß, H.-O. KARNATH, B. DE HAAN;
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Abstract: Visual extinction is a neuropsychological deficit that can follow unilateral, most commonly right hemispheric, brain damage. Extinction patients are able to detect contra- and ipsilesionally presented single targets, but fail to report contralesional targets when an ipsilesional target is concurrently presented. The existing literature suggests a critical role for both the right temporo-parietal junction (TPJ) and the right intraparietal sulcus (IPS) in the ability to attend and respond to multiple targets simultaneously, however, the precise role of each of these areas in this ability is currently unclear. In this study we combined the Theory of Visual Attention (TVA) with continuous theta burst stimulation (cTBS) in neurologically healthy subjects to directly investigate the role of the right TPJ and IPS in attentional selection in multi-target environments. Specifically, we investigated the TVA processes impaired after temporary inhibition of neural activity at either the right TPJ or the right IPS and the relation of these TVA processes to the ability to attend and respond to multiple targets simultaneously. Additionally, we measured the effect of cTBS on the motor evoked potential (MEP) in each individual subject to obtain an individual estimate of the strength of cTBS inhibition. Our results suggest that cTBS to the IPS modulated top-down attentional selection. However, cTBS to the TPJ failed to induce a change in TVA parameters. We conclude that the IPS plays a crucial role in top-down attentional control, while the function of TPJ remains unclear. This suggests that the IPS may modulate attention in multi-target environments and its failure in extinction via top-down prioritization of targets over distractors.

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Title: Attention alters animal and action representation in highly-distributed, functionally-defined cortical parcels

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Italy; [4]Dept. of Medicina Specialistica, Diagnostica e Sperimentale (DIMES), Med. Sch., Univ. of Bologna, Bologna, Italy

Abstract: Attending to particular semantic features of a complex, naturalistic stimulus may require attentional mechanisms to operate across highly-distributed neural populations. Here we apply simple models of representational space to extensive, functionally-defined cortical parcels to evaluate how attention reshapes the distributed neural representation of animal and action categories. Functional MRI was used to measure neural responses while participants viewed brief naturalistic video clips of animals behaving in their environments. Stimuli comprised five different folk animal taxa (primates, ungulates, birds, reptiles, insects) each performing four different actions (eating, fighting, running, swimming) in a fully-crossed design for 20 total conditions. In each run, participants performed a 1-back task requiring them to selectively attend to either the animal or action types. Whole-brain surface-based searchlight hyperalignment based on neural responses to a 1 hr nature documentary was used to rotate participants' response patterns into representational correspondence. Instead of relying on anatomical boundaries or functional localizers to constrain our multivariate analyses, we used an unsupervised learning algorithm to cluster cortical searchlights based on shared representational geometry. We cross-validated cluster solutions across participants to identify particularly stable parcellations at several scales. We then used representational similarity analysis to evaluate how the similarity between response patterns for the 20 conditions within each parcel related to several target similarity structures. We found that attending to action information increases the discriminability of action category representations in intraparietal and pericentral cortices. Attending to animal information increased animal category discriminability in ventral temporal cortex. These effects were driven by both decreased within-category distances and increased between-category distances. For both action representation in the dorsal visual pathway and animal representation in the ventral pathway, representation was more robust in earlier visual areas when attention was directed elsewhere, suggesting that attention selectively increases depth of processing. The action category model better fit neural representation than did animal category and animacy continuum models in most cortical parcels. Surprisingly, this advantage was also quite large in ventral temporal cortex, suggesting that the representation of animal behavior may eclipse that of animal form in the ventral visual pathway in the context of naturalistic vision.

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Title: Semantic relationships of real-world objects bias visual attention

Authors: *G. L. MALCOLM[1], C.-I. NAH[2], S. SHEREMATA[3], S. SHOMSTEIN[2];
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Abstract: We primarily attend to objects when observing the environment, making object properties an integral component in constraining attentional allocation. While extensive research has investigated object properties in relation to space and low-level features, recent evidence from our lab suggests that high-level, semantic relationships between objects influence allocation of attentional resources, even when these relationships are task-irrelevant. Behavioral results found that, when three objects were presented, attention was biased to the semantically related object (Malcolm & Shomstein, 2014). Here, we investigated the neural mechanisms that prioritize semantic relationships between real-world objects to bias attentional allocation. We specifically focused on investigating whether early visual cortex (areas V1-V4) and object-sensitive lateral occipital cortex (LOC) showed semantic-based modulation of sensory response. Additionally, we examined whether retinotopically organized regions within the inferior parietal sulcus, areas IPS0-2, are the regions responsible for eliciting early sensory modulations consistent with semantic relatedness. Participants viewed three objects arranged in a triangle, or 'triad', with one object just above fixation and one in each periphery, below the midline. Critically, one of the peripheral objects was always semantically related to the central object while the other was not (e.g., if the central object was a lamp, one peripheral item was a light bulb and the other an envelope). After the triads remained on the screen for an average of 4s, a target along with two distractors appeared (one on each object). Targets occurred on all three objects equally, making the semantic relationship between the objects irrelevant to the task. Participants were asked to discriminate the target while keeping their eyes fixated on a central cross. We observed that neural activity was biased toward the semantically related object throughout visually responsive cortex. Importantly, semantic based modulation of visual responses was observed despite the fact that semantic relationships were task-irrelevant. The results demonstrate that semantic relationships between objects modulate visual cortical activity even at the earliest stages, biasing spatial attention.

Disclosures: G.L. Malcolm: None. **C. Nah:** None. **S. Sheremata:** None. **S. Shomstein:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 79.19/V28

Topic: F.01. Human Cognition and Behavior

Title: Alpha oscillation as a clock for visual processing -Illusory jitter frequency correlated with individual alpha frequency-

Authors: *S. MINAMI[1,2], K. AMANO[2,1];
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Abstract: Introduction: Recent studies have demonstrated the possible involvement of alpha oscillations (8-13 Hz) in visual processing (Jensen et al., 2012). To further elucidate the functional role of alpha oscillations, we utilized an illusion called motion-induced spatial conflict (Arnold and Johnston, 2003). In this illusion, a moving isoluminant border placed in close proximity to moving luminance-defined borders appears to jitter. In a previous study, we found that the frequency of illusory jitter perception (~10 Hz) matches that of neural oscillations whose amplitudes are modulated during jitter perception. However, a causal link between alpha oscillations and illusory jitter perception has not been demonstrated. Here, we tested the hypothesis that alpha oscillations contribute to the generation of illusory jitters by examining inter-individual differences in peak alpha frequency (PAF) and perceived jitter frequency. Methods: PAF was measured during the resting state using magnetoencephalography (MEG). Subjects opened and closed their eyes in response to a cue sound in a dark room. In a separate psychophysical experiment to measure perceived jitter frequency, subjects judged whether illusory jitter stimuli in the upper visual field jittered faster than a physical jitter stimulus at a random frequency (4-13 Hz). The perceived jitter frequency was defined as the frequency at which the response rate was 50%. Results and Discussion: We found that the perceived jitter frequency of each subject was highly correlated with the PAF during the resting state, regardless of eye-opening. Given that an illusory jitter stimulus was not presented during the alpha frequency measurement, the PAF was unaffected by illusory jitter perception, and reflects the intrinsic alpha frequency of each subject. Therefore, the correlation between the perceived jitter frequency and PAF suggests that alpha oscillations are involved in the generation of the illusory jitter perception. The PAF during the perception of illusory jitter also correlated with the perceived jitter frequency, indicating that the intrinsic alpha is sustained during jitter perception. The perceived jitter frequency did not correlate with the peak frequency of other oscillations including beta. Based on these findings, we propose that alpha oscillations act as a clock for visual processing. In the case of illusory jitters, dissociation between motion-based delayed

position representation in the dorsal stream and the object-based, physically correct position representation is resolved at the alpha frequency.

Disclosures: **S. Minami:** None. **K. Amano:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

Location: Hall A

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Program#/Poster#: 79.20/V29

Topic: F.01. Human Cognition and Behavior

Support: NIMH 2R01MH087214-06A1

Title: Reconstructing spatial attention maps from EEG alpha activity

Authors: ***J. J. FOSTER**[1], D. W. SUTTERER[1], J. T. SERENCES[2,3], E. K. VOGEL[1], E. AWH[1]; [1]Dept. of Psychology, Univ. of Oregon, Eugene, OR; [2]Dept. of Psychology, [3]Neurosciences Grad. Program, UCSD, San Diego, CA

Abstract: Past work has demonstrated a clear link between oscillatory neural activity in the alpha band (8-13Hz) and covert spatial attention. For example Rihs, Michel, and Thut (2007) found that the topographic distribution of alpha activity across the scalp covaried with attended location in a spatial cueing task. Here, we show that alpha topography - measured using electroencephalography (EEG) - can be used to precisely track the locus of covert attention in a time-resolved fashion. We used an encoding model to reconstruct spatially-specific population response profiles following the onset of central cues that prompted endogenous shifts of spatial attention. In Experiment 1, a central cue (87.5% valid) directed participants to one of eight placeholders arranged in a circle around fixation. After 1250 ms, a search array was presented and participants were asked to find the digit among letters. Robust enhancements of target discrimination at the attended locations confirmed the efficacy of the central cues. We observed robust spatially-specific tuning functions (TFs) that tracked the covertly attended locations. These TFs emerged 400-500 ms after cue onset, a time-course that dovetails with past behavioral and neural estimates of the latency for endogenous spatial orienting. We observed the same empirical pattern with both physically-congruent spatial cues (oriented bars indicating the cued position) and symbolic cues (letters and digits) that had no pre-experimental association with the cued positions. A further control experiment ruled out biases in eye position as a potential source of this spatially-specific neural signal. Together, these results highlight the role that alpha activity plays in the deployment of covert spatial attention, and suggest that alpha-based TFs

provide a robust approach for tracking the deployment of spatial attention with excellent temporal resolution.

Disclosures: **J.J. Foster:** None. **D.W. Sutterer:** None. **J.T. Serences:** None. **E.K. Vogel:** None. **E. Awh:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

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Program#/Poster#: 79.21/V30

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant AG044862

NSF Grant BCS-1439188

Title: Task-dependent change of alpha oscillation frequency and its functional significance

Authors: ***I. BABU HENRY SAMUEL**, C. WANG, M. DING;
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Abstract: Neuronal oscillations are characterized by amplitude and frequency. In the case of human alpha oscillations (8 to 12 Hz), whereas the modulation of alpha amplitude by task conditions have been extensively studied, the frequency of alpha in a given individual is often considered a trait-level variable. Task dependent changes of alpha frequency and its functional relevance has not been fully established. We addressed this problem by recording high-density EEG from subjects performing a modified Sternberg working memory task. In this task the subject was shown a set of digits (0 to 9) on a CRT monitor for 1s (encoding) which was followed by a 3s retention period. At the end of retention a probe digit was presented and a “yes” (index finger in the dominant hand) or “no” (middle finger) button press was required to indicate whether the probe digit belonged to the set. Memory load was controlled by the size of the digit set which in this experiment was chosen to be 1, 3 or 5. We showed that (i) Alpha Frequency (AF) is modulated by memory load, (ii) the load modulation is different across different functional states (encoding versus retention), and (iii) high alpha frequency is associated with slower reaction times and vice versa. There is also evidence indicating that the visual p1 evoked component has smaller amplitude and higher latency when the pre-probe AF is high. These results suggest that alpha frequency is a task dependent variable and its increase signals

increased inhibitory activity. Finally, attempts were made to explain alpha frequency modulation within the two-alpha (high versus low alpha) framework.

Disclosures: **I. Babu Henry Samuel:** None. **C. Wang:** None. **M. Ding:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 79.22/V31

Topic: F.01. Human Cognition and Behavior

Title: Modulation of visual perception by low frequency oscillations

Authors: ***S. NELLI**, J. SERENCES;
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Abstract: Selective attention can prioritize the processing of behaviorally relevant visual stimuli. Oscillatory modulation of neural excitability may play a role in this ability by altering information processing both within early visual areas and between early visual areas and downstream regions. One putative oscillatory mechanism of attentional control is anticipatory desynchronization of EEG alpha rhythms (8-12 Hz) in visual cortex. Decreases in alpha power in task-related regions may result in enhanced task performance by allowing increased neural firing. In addition, prestimulus differences in alpha phase have also been associated with task performance, suggesting differential information processing at separate phases of the alpha cycle. Together these findings support the hypothesis that alpha oscillations affect perception through rhythmic inhibition of cortical activity. However, it is not clear if alpha oscillations play a role in directly regulating the quality of sensory processing, or if alpha oscillations impact behavior by altering the efficacy of information transfer from early to later stages of processing. Here we examined the impact of alpha oscillations on behavioral performance in a discrimination task in which subjects had to report the orientation of a temporally unexpected and briefly flashed (~8 ms) orientated grating. The contrast of this grating was determined before the start of the experiment for each subject so that accuracy was fixed between 60% and 65%. Performance on this task was then evaluated as a function of alpha power and alpha phase (as assessed with scalp EEG). We observed that alpha activity during the post-stimulus period strongly predicted behavior, consistent with a role for alpha in mediating the relay of sensory signals to downstream areas.

Disclosures: **S. Nelli:** None. **J. Serences:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Support: MEXT KAKENHI 25240019

MEXT KAKENHI 26119516

Title: EEG phase shift caused by visual-spatial attention accompanies ventriloquism effect

Authors: T. KUMAGAI, *H. MIZUHARA;
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Abstract: The brain, in nature, accepts and integrates different information sources. However, multisensory spatial information is not always combined correctly. One example is the "ventriloquism effect," which is the illusion whereby we misunderstand the source of a sound, attributing it, as it were, to the dummy rather than to his human partner. The neural basis of this effect remains an open question. In the present study, we examined the ventriloquism effect according to the hypothesis that visual-spatial attention causes an electroencephalographic (EEG) phase shift in the auditory cortex, indicating that auditory attention is critical to the input of auditory position. Our research applied exogenous or endogenous visual spatial attention as the main factor behind the ventriloquism effect. We used sound stimuli which lent themselves to virtual location by interaural time difference, and employed a two-alternative forced-choice paradigm. After looking at salient visual stimuli, participants were asked whether the sound source was located on their left or their right side. We obtained EEG measurements while participants conducted the localization task. The behavioral task showed that the sound position was preferentially detected as if originating from the position to which exogenous or endogenous visual attention was attracted. In the exogenous attentional condition, EEG oscillation in the theta frequency range was more likely phase-coupled for the ventriloquism effect. The topography showed the phase-locking at the contralateral occipital/temporal electrodes site in the visual-cue hemifield. On the other hand, we found no laterality of EEG phase-locking in the non-ventriloquism-effect condition. Moreover, in the endogenous spatial-attentional condition, we did not find any laterality of phase-locking with or without the ventriloquism effect. This result suggested the following: (1) that upon exogenous visual-spatial attention, the phase shift arising from the visual cortex is transmitted to the ipsilateral auditory cortex, and thus the visual-spatial-attention-enacted auditory space added to the visual space causes the ventriloquism effect; (2)

that endogenous visual-spatial attention induces the ventriloquism effect without the phase shift of exogenous attention.

Disclosures: **T. Kumagai:** None. **H. Mizuhara:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: DFG (SFB936/A3/B6)

EU (ERC-2010-AdG-269716)

Title: Attention modulates large-scale synchronization of low-frequency oscillations in multisensory processing

Authors: ***J. DAUME**[1], U. FRIESE[1], F. GÖSCHL[1], P. WANG[1], P. KÖNIG[2], A. K. ENGEL[1]; [1]Dept. of Neuro- and Pathophysiology, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [2]Inst. of Cognitive Sci., Osnabrück Univ., Osnabrück, Germany

Abstract: Directing attention towards relevant multisensory events is crucial to cope with the vast amount of information reaching our sensory systems. However, the mechanisms underlying attentional modulations of information processing remain unknown. Here, we used MEG to study large-scale synchronization across distant brain areas as a putative mechanism by which attention might modulate the processing of congruent or incongruent audiovisual stimuli. Healthy participants were presented with stimuli composed of a circular drifting grating accompanied by a complex sound. On every trial, brief simultaneous near-threshold changes in sound volume and visual contrast occurred twice during the 2.5 seconds lasting presentation. Each of these changes was either congruent (increase or decrease in both modalities) or incongruent (increase in one modality and decrease in the other modality). Prior to stimulation a cue indicated whether the first or the second multisensory stimulus change would be task-relevant. Participants were instructed to specify whether both modalities varied congruently or incongruently for the attended change. For the analysis of oscillatory brain activity we focused on a 400 ms time window following the first amplitude change. Spectral power in source space was examined using beamformer algorithms, and revealed strong influences of attention on low- and high-frequency oscillations. Gamma-band power was increased for attended versus

unattended bimodal changes in visual and auditory cortices as well as parietal and frontal brain regions. Significant decreases in power related to the allocation of attention were observed across posterior cortex regions in the beta-, alpha-, and theta-band. Analyses of functional connectivity revealed that attention was associated with increased theta-band coherence in right frontopolar cortex, bilateral inferior frontal cortex and in bilateral temporal regions. Attention also led to decreased alpha-band coherence in bilateral occipital and parietal cortex. Finally, beta-band coherence was lower for congruent as opposed to incongruent bimodal stimulation in mid-cingular cortex and right superior temporal cortex. Our results demonstrate that directing attention towards bimodal events modulates oscillatory activity in large-scale cortical networks comprising early auditory and visual as well as multisensory areas. Notably, during attended multisensory processing low-frequency coherence increased in a fronto-temporal network. These findings indicate that large-scale synchronization might orchestrate the functional integration in multisensory brain networks.

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Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 79.25/V34

Topic: F.01. Human Cognition and Behavior

Title: Neural oscillations in temporal pole: local and global network in a-synchronous audio-visual speech matching task

Authors: ***T. OHKI**[1,2], A. GUNJI[3], Y. TAKEI[4], H. TAKAHASHI[5], Y. KANEKO[6], Y. KITA[5], N. HIRONAGA[8], S. TOBIMATSU[8], M. INAGAKI[7], K. HIRAKI[9];
[1]Grad. Sch. of Arts and Sci., Univ. of Tokyo, Tokyo, Japan; [2]Dept. of Developmental Disorders, Natl. Inst. of Mental Health, Natl. Ctr. of Neurol. and Psychiatry, Saitama, Japan; [3]Col. of Educational and Human Sci., Yokohama Natl. Univ., Yokohama, Japan; [4]Dept. of Psychiatry and Neurosci., Gunma Univ. Grad. Sch. of Med., Gunma, Japan; [5]Dept. of Child and Adolescent Mental Hlth., [6]Dept. of Neurosurg., [7]Dept. of Developmental Disorders, Natl. Inst. of Mental Hlth., Natl. Ctr. of Neurol. and Psychiatry, Saitama, Japan; [8]Grad. Sch. of Med. Sci., Kyushu Univ., Fukuoka, Japan; [9]Grad. Sch. of Arts and Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: Multisensory process is an ability to combine cues from various modalities, and a basic feature of brain function. Such a process directly contributes to forming perception. To

reveal multisensory processes occur in unisensory stage, recent studies focus on onset mechanism. In the next step, therefore, how and where sustainable multi-sensory processes are implemented should be answered. To investigate this issue, interpreting “the cocktail-party effect” in a broad sense, we designed an audio-visual speech matching task, and investigated detailed binding mechanism, using magnetoencephalography (MEG). In our task, two films were presented simultaneously, which consisted of two different sentences spoken by the identical person. To accomplish our task, participants were required to attend to not only auditory stimuli but also mouth movement persistently. We acquired MEG data during the task using a 306-ch whole-head MEG (Neuromag) from 15 healthy volunteers. First, source estimation analysis showed persistent activations in temporal pole (TP). We used wavelet transform for source data of TP, which revealed the activations consisted of multiple frequency components, especially delta (3-5Hz) and beta (15-30 Hz). To examine these frequency bands coordinate, we quantified phase amplitude coupling (PAC), which revealed a novel PAC pattern between delta phase and beta amplitude in TP (Fig. 1). Finally, measuring coherence, we confirmed TP operated together with motor area, especially the post-central area by the medium of delta (Fig. 2). In short, these results can be interpreted as following: previous studies report TP is a convergent hub of somatosensory, visual, auditory and language network. Visual and auditory information might be converged in TP. Second, delta is reported as one of the most adequate oscillations for encoding speech information, and beta as motor related oscillations. To support this idea, we confirmed TP coordinated with motor area. Thus, we propose PAC in TP is a part of mechanism to integrate audio-visual information.

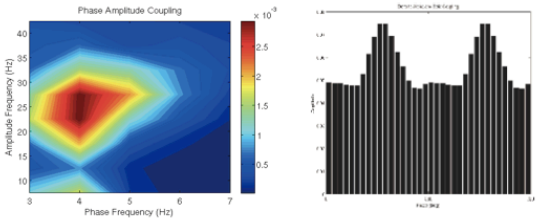

Figure 1. Phase-amplitude comodulogram. In left, vertical line denotes amplitude, horizontal line does phase. In right, Bars indicate delta phase bins from 0-720 degree. Therefore, each bin denotes 20 degree respectvely.

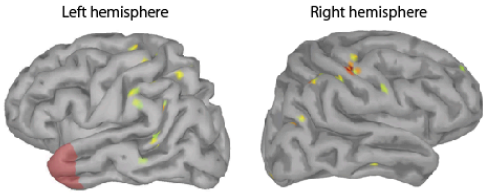

Figure 2. Imaginary coherence. Significant clusters tend to overlap with parietal lobe especially the post-central areas bilaterally. Red color denotes TP in the left hemisphere. This region is used as the seed region in calculating coherence values.

Disclosures: **T. Ohki:** None. **A. Gunji:** None. **Y. Takei:** None. **H. Takahashi:** None. **Y. Kaneko:** None. **Y. Kita:** None. **N. Hironaga:** None. **S. Tobimatsu:** None. **M. Inagaki:** None. **K. Hiraki:** None.

Poster

079. Human Cognition and Behavior: Functional Mechanisms of Attention

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 79.26/V35

Topic: F.01. Human Cognition and Behavior

Support: NSF 3-GEN0377

Title: The representational similarity of face morphs predicts performance in an independent visual search task

Authors: *J. LEE, J. J. GENG;
Ctr. For Mind and Brain, UC Davis, Davis, CA

Abstract: The representation of task-related information determines the speed of attentional selection and distractor suppression. However, it remains unclear how the neural representation of stimulus similarity affects attentional processes. We investigated this problem by examining the relationship between brain regions that encode categorical representations of face identity and performance in an independent visual search task. Functional magnetic resonance imaging (fMRI) data were acquired during a task in which face morphs between two famous face identities were classified as one or the other person. In addition, a house or a scrambled face image was sometimes presented and required the third "non-face" response. Representational similarity analysis (RSA) was used with a whole-brain searchlight procedure to identify regions with patterns of activation that correlated with identity classification (using the Spearman rank correlation coefficient). The results confirmed that face selective areas in the ventral temporal cortex and inferior occipital gyrus contained highly similar patterns of activation for all faces, which were dissimilar from all non-face stimuli. However, patterns of activation in the right middle frontal gyrus and the left inferior parietal cortex were highly consistent with the classification of face identity. Interestingly, performance in an independent visual search task in which one of the face identities served as the target and the various face morphs appeared as distractors was predicted by the behavioral and neural patterns during the classification task: reaction times to select the target increased as the classification similarity between the target and the distractor morph increased. These results suggest that the contents of the attentional template, which determine the speed of attentional selection, reflects the categorical structure of target stimuli that is represented in frontal and parietal areas as well as in stimulus-specific perceptual areas.

Disclosures: J. Lee: None. **J.J. Geng:** None.

Poster

080. Human Cognition: Control and Flexibility

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 80.01/V36

Topic: F.01. Human Cognition and Behavior

Title: Effortful control relates to intrinsic functional connectivity in dlPFC and dACC

Authors: *N. THAI, B. C. TABER-THOMAS, M. MAGGI, K. E. PEREZ-EDGAR, P. M. COLE;
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Abstract: Effortful control, as measured through self-reports developed by Rothbart and colleagues (2001), has been studied in relation to many important developmental achievements. For example, effortful control is positively related to success in school, higher levels of empathy and prosocial behaviors, greater ability to resolve conflict, and lowered risk for pathologies such as conduct disorder, substance abuse and antisocial behavior (Ellis et al., 2004; Rothbart et al., 1994). During adolescence, the structure and activity of the anterior cingulate has been found to correlate with self-reports of effortful control (Posner et al., 2007). Resting state, or task-free analysis of intrinsic functional connectivity (iFC), may help elucidate broader neural architectures that support effortful control in children. iFC in executive control and salience networks has been associated with emotional functioning (e.g., internalizing and externalizing problems), as well as with behavioral traits (e.g., empathy) (Vaidya & Gordon, 2013). iFC continues during sleep and does not require active cognitive processes (Fukunaga et al., 2006). The current study investigates links between children's intrinsic functional connectivity and the temperamental trait of effortful control. 16 children (9M) ages 6-10 years (MAge = 8.35 SDAge = 1.37) provided resting state data during sleep. A voxelwise measure of global iFC was obtained for each participant by computing the average connectivity of each voxel with all other voxels in the brain. These maps were then entered into a whole-brain regression analysis to reveal regions where effortful control related to global iFC (cluster corrected p FDR < 0.05, with voxelwise $p < 0.005$). Effortful control was positively related to global iFC in left dlPFC and dACC. These findings are consistent with the extant literature on iFC relating to executive functions. Posner and Fan (2008) have identified an executive network, consisting of the anterior cingulate and prefrontal cortex, which is involved in resolving conflict among response tendencies. Other research has supported the cognitive role of the ACC in processing errors and conflict (Kerns et al., 2004; Menon et al., 2001). Effortful control, in turn, facilitates error and conflict monitoring. Intrinsic connectivity involving dlPFC has been associated with executive

attention (Posner & Rothbart, 2009). Taken together, we found support for individual differences in intrinsic connectivity involving dlPFC and dACC to be associated with effortful control, implicating that effortful control engages both the central executive (anchored by dlPFC) and salience (anchored by dACC) networks.

Disclosures: **N. Thai:** None. **B.C. Taber-Thomas:** None. **M. Maggi:** None. **K.E. Perez-Edgar:** None. **P.M. Cole:** None.

Poster

080. Human Cognition: Control and Flexibility

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Program#/Poster#: 80.02/V37

Topic: F.01. Human Cognition and Behavior

Support: USA MRMC Contract W81XWH-14-C-0018

Title: Monitoring, extracting, and encoding indicators of cognitive workload(MEDIC)

Authors: ***B. K. BRACKEN**[1], N. PALMON[2], B. D. FREDERICK[3], N. J. COOKE[4], V. ROMERO[2], D. KOELLE[2], J. PFAUTZ[2];
[1]Cognitive Systems Div., [2]Charles River Analytics, Cambridge, MA; [3]McLean Hospital/Harvard Med. Sch., Belmont, MA; [4]Arizona State Univ., Mesa, AZ

Abstract: Background: The success of emergency medical personnel in saving lives depends on their acting quickly and effectively, as individuals and as teams. Training must go beyond individual skills to include team member interactions, and how skills transfer to stressful environments. Currently, trainers must infer competence by observation alone_a challenging task. Automatically sensing indicators of cognitive workload can augment performance observations, offering insight into factors underlying that performance. Method: We designed and demonstrated a system to augment training by Monitoring, Extracting, and Decoding Indicators of Cognitive workload (MEDIC). MEDIC combines a multimodal suite of unobtrusive, field-ready neurophysiological, physiological, and behavioral sensors; complex event processing to extract and fuse the best indicators of cognitive workload and team dynamics from the multiple, high-volume data streams originating from the sensor suite; and probabilistic modeling to interpret those indicators for easy understanding. Results/Conclusions: We designed a head-mounted device to measure cognitive load, a body band device to measure physical activity, and a user interface (UI) for trainers to take notes and record time-tagged photos and videos as the scenario unfolds. During our pilot study, we successfully extracted and fused data

from two individuals across both physically (simulated CPR) and cognitively (arithmetic and n-back cognitive task) demanding conditions. We concisely identified complex sequences of events in raw data using complex event processing. MEDIC is capable of processing both continuous (heart rate, oxygenated hemoglobin) and discrete (communication events, trainer observations on performance) variables. We explored five methods of data modeling, and determined that for our pilot data weighted averaging was most accurate for estimating individual state (stress and cognitive workload), but Causal Influence Models were most accurate for team state (team dynamics). Finally, we designed a separate UI that displays interpreted data after the simulation, including information about each state for each individual and for the team. Future work will include validation of each component of MEDIC using laboratory studies and testing in realistic training environments. This work was supported by the U.S. Army Medical Research and Material Command under Contract No. W81XWH-14-C-0018. The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

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Poster

080. Human Cognition: Control and Flexibility

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Program#/Poster#: 80.03/V38

Topic: F.01. Human Cognition and Behavior

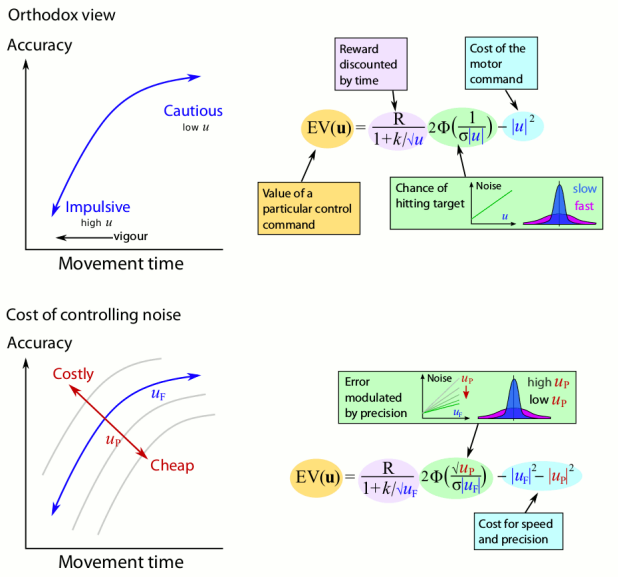
Support: Wellcome Trust Grant WT 098282

Title: Reward pays the cost of noise reduction in cognitive and motor control

Authors: *S. G. MANOHAR[1,2], K. MUHAMMED[2], M. HUSAIN[2];
[1]Dept. of Exptl. Psychology, Oxford, United Kingdom; [2]Nuffield Dept. of Clin. Neurosciences, Univ. of Oxford, Oxford, United Kingdom

Abstract: The speed-accuracy trade-off is a fundamental principle in animal and human behaviour. Classically, this is explained by an optimal balance between the benefits of obtaining a goal sooner, and the disadvantages of speed, e.g. energetic cost and lower signal-to-noise ratios. However, recent reports suggest that motivation by reward can break the orthodox trade-off. We studied this with an incentivised speeded distractor-avoidance task. We found that

reward improved cognitive control over distraction, but simultaneously reduced reaction time. In the motor domain, reward increased both the velocity and accuracy of movements. To account for these effects, we consider a new factor, the cost of control. Mathematically, we propose that there is a fixed cost for a proportional increase in the signal-to-noise ratio. We suggest that, similar to motor control signals, a precision signal that attenuates noise might carry a cost. For example in rise-to-threshold models of decision-making, the noise might be attenuated relative to signal, but this gain incurs an economic cost that must be weighed against the benefits. The cost of control model fitted the data better than models without this component. Since rewards are signalled by dopamine, which is also thought to play a part in motivating effortful behaviour, we hypothesised that states of dopamine depletion may exhibit an increased cost of control. To test this, 19 patients with Parkinson's disease, a condition associated with dopamine depletion, were tested on the oculomotor task. The effects of reward were weaker in these patients, and within the model corresponded to an increased cost of control. Further, the model predicted that larger commands, being subject to greater noise, will be more amenable to noise reduction by reward. To test this, we varied the amplitude of saccades. High rewards increased both speed and accuracy, but reward effects were stronger for larger movements. This supports the hypothesis that signal-to-noise ratios can be improved at a cost, and that this cost applies proportionally to the amount of noise present.



Disclosures: **S.G. Manohar:** None. **K. Muhammed:** None. **M. Husain:** None.

Poster

080. Human Cognition: Control and Flexibility

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 80.04/V39

Topic: F.01. Human Cognition and Behavior

Support: JSPS KAKENHI 24240041

Title: Inferior parietal lobules plays an important role in individual differences in executive function: a study with fMRI and SNP

Authors: *Y. UEDA[1], Y. KIKUNO[2], H. YAMAMOTO[1], J. SAIKI[1];
[1]Kyoto Univ., Kyoto, Japan; [2]Nagasaki Univ., Nagasaki, Japan

Abstract: Focusing on relevant information with ignoring irrelevant (distracting) information is an important skill to facilitate an efficiency of our behavior. This skill is called executive function. This function is realized with co-activation of cingulo-frontal-parietal cognitive/attention network (CFP network; Bush and Shin, 2006) in brain and has large individual differences. In this study, we replicated their experiment and investigated brain regions whose activity was correlated with interference task performance to reveal which brain regions contribute to individual differences in executive function. Furthermore, we also investigated the relationship between brain activations and single nucleotide polymorphisms in the dopamine receptor D4 gene (DRD4 rs1800955) and acetylcholine receptor subunit α-4 gene (CHRNA4 rs1044396) to reveal the mechanisms of the individual differences. In the experiment, 96 participants underwent the multi-source interference task (Bush and Shin, 2003, 2006), in which they were asked to identify the oddball digit among three digits with a button press. In the control condition, position of the oddball digit was congruent with its response position on the button box whereas in the interference condition, it was incongruent with its position on the box. Moreover, in the interference condition, the distractor digits were chosen randomly from the oddball targets in other trials. The results indicated that CFP network showed robust activity in the interference condition than in the control condition. Notably, brain activity correlated with the task performance was found in the left dorsolateral prefrontal cortex and the bilateral inferior parietal lobules (IPLs). In these regions, activations were stronger for participants who showed longer reaction times in the interference condition. Regarding to the gene-cognition relationships, participants with the TT and CC genotype in DRD4 and CHRNA4, respectively, showed reduced interference from distractors than other genotyped participants. Factorial analysis controlled with task performance revealed that their activities in bilateral IPLs and thalamus were significantly increased during the task than other genotyped participants. Altogether, these findings suggest that individual differences in executive function may depend on the susceptibility of these regions and/or their connectivity, which in turn were controlled by DRD4 and CHRN4.

Disclosures: **Y. Ueda:** None. **Y. Kikuno:** None. **H. Yamamoto:** None. **J. Saiki:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: Gates Foundation

NICHD 5F32HD079143-02 to APM

Title: Development of functional networks supporting delay of gratification in young children

Authors: *A. T. PARK, P. K. SAXLER, A. B. CYR, J. D. E. GABRIELI, A. P. MACKEY; Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Delay of gratification during early childhood has been linked to important future academic outcomes. Although the developmental trajectory of delay of gratification has been examined at the behavioral level, few neuroimaging studies have been done with young children to investigate the nature and development of the functional networks underlying these behavioral differences. In this study, 36 participants between the ages of 4 and 7 performed a delay of gratification task (the "marshmallow test") and completed structural and resting state fMRI (rs-fMRI) scans. 23 participants were able to delay gratification (6.5 years, 9 males), i.e., wait for the larger reward, and 13 participants were not able to wait (5.8 years, 8 males). The groups differed by age ($t(34) = 2.02$, $p = .05$) but not gender ($\chi2(1, N = 36) = 1.7$, $p = .2$). Rs-fMRI preprocessing steps included: realignment, slice timing correction, spatial smoothing, bandpass filtering (.01-.1 Hz), physiological noise correction (aCompCorr), and outlier detection and regression (ART). The groups did not differ in number of motion outliers (delayers: 3.5, non-delayers: 4.2, $Z = .25$, $p = .8$). We chose inferior frontal gyrus pars opercularis (IFG) as a seed due to its involvement in delay of gratification in adults. IFG was defined from each participant's individual structural scan (FreeSurfer 5.3), and registered to functional space. Group-level analyses controlled for number of motion outliers. Controlling for age, children who were able to wait showed significantly less connectivity between left IFG and reward-related regions, the ventral striatum and substantia nigra. Delayers also showed significantly greater connectivity between left IFG and left precentral gyrus. Taken together, these results suggest that successful delay of gratification in early childhood is linked to differences in functional connectivity between cognitive control areas and brain regions responsible for processing rewards and initiating actions. The parallels between our results and fMRI findings in adults 40 years after performance of the "marshmallow task" (Casey et al., PNAS, 2011) suggest that individual

differences in these functional networks may arise early in development and persist into adulthood in the absence of targeted intervention. Future work will investigate the behavioral and neural impacts of educational programs that aim to improve cognitive control in young children.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: Student Blugold Commitment Differential Tuition funds through the University of Wisconsin-Eau Claire Summer Research Experiences for Undergraduates program

Title: Influence of gender performance stereotype on the error-related negativity

Authors: *D. S. LELAND, K. A. ROLEFSON, S. E. BADOUR, G. L. BYE, C. M. BRENNAN, R. S. BELOTT;
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Abstract: Gender stereotype threat refers to the fear of confirming the negative stereotypes of one's gender. We predict that the salience of task errors will be influenced by participants' expectations regarding which gender generally performs better on the task. We are testing this using the flanker task, where participants indicate the direction of a central arrow in a series of five arrows. We have replicated classic findings of an error-related negativity (ERN; a frontocentral negativity larger when evoked by errors than correct responses) and will present preliminary findings on how the ERN is modulated by gender performance stereotype. We predict that when participants are told that their gender (as opposed to the opposite gender) has a performance advantage for the flanker task, they will be more concerned about errors and thus generate larger ERNs.

Disclosures: D.S. Leland: None. **K.A. Rolefson:** None. **S.E. BaDour:** None. **G.L. Bye:** None. **C.M. Brennan:** None. **R.S. Belott:** None.

Poster

080. Human Cognition: Control and Flexibility

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Program#/Poster#: 80.07/V42

Topic: F.01. Human Cognition and Behavior

Title: Mindfulness-of-breathing exercise affects eeg alpha-power measures of self-monitoring

Authors: H. BING-CANAR, J. PIZZUTO, *R. COMPTON;
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Abstract: The present study assessed how a brief mindfulness induction affects neural markers of self-monitoring and task engagement during a speeded cognitive performance task. Participants (n = 33) were assigned to either engage in a 14-minute mindfulness-of-breathing audio exercise or attend to a control listening exercise. They then completed a six-choice Stroop task of selective attention while EEG was recorded. EEG measures of performance monitoring included quantification of alpha power in the 1280-ms inter-trial interval following button-press responses. Prior evidence has found transient but robust increases in alpha power following correct responses and suppressed alpha following error responses, suggesting a pattern of "mental relaxation" after correct responses specifically (Carp & Compton, 2009). In the present study, participants in the mindfulness condition demonstrated this effect to a greater extent than controls. Alpha power during the window of stimulus processing did not differ between mindful versus control groups, arguing against the possibility of generally increased alpha power consistent with an overall relaxation effect. Instead, the results suggest that alpha power changed more dynamically across trial events in the mindful participants, such that greater alpha power (and greater inferred mental disengagement) was evident during brief "relaxation" periods between trials in the mindful group while at the same time stimulus processing and error processing evoked engagement that was equivalent across groups. Event-related potential markers of error-monitoring, including the error-related negativity and error positivity, were not affected by the mindfulness manipulation but were predicted by individual differences in self-reported mindful awareness and anxiety. Together the results suggest that a mindfulness-of-breathing exercise may promote more dynamic patterns of engagement during task performance.

Disclosures: H. Bing-Canar: None. **J. Pizzuto:** None. **R. Compton:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant DA026452

Title: Withholding a reward-driven action: studies of corticospinal dynamics and the effect of cognitive depletion

Authors: *S. FREEMAN, D. LU, A. ARON;
UCSD, San Diego, CA

Abstract: Controlling an inappropriate response tendency in the face of a reward-predicting stimulus likely depends on the strength of the reward-driven activation, the strength of a putative top-down control process, and their relative timing. We developed a rewarded Go/NoGo paradigm to investigate such dynamics. Participants made rapid responses (on Go trials) to high versus low reward-predicting stimuli and sometimes had to withhold responding (on NoGo trials) in the face of the same stimuli. Behaviorally, for high vs. low reward stimuli, responses were faster on Go trials ($p<0.001$) and there were more errors of commission on NoGo trials ($p<0.001$)_showing that the value of the stimulus modulated response prepotency even on trials where responses were not permitted. We used single-pulse Transcranial Magnetic Stimulation to map out the corticospinal excitability dynamics. We found that, for NoGo trials, there was an early rise in motor activation that was then sharply reduced beneath baseline. This activation-reduction pattern was more pronounced for high versus low reward trials and in participants with greater motivational drive for reward (determined by their average response times). Those participants who had relatively more activation compared to reduction also made more NoGo errors ($r=0.44$, $p=0.02$). These results suggest that an effortful control process 'kicks in' to mitigate early reward-related response activation. A follow-on experiment tested whether the reduction phase reflects such a top-down control process that is related to the strength of the preceding activation and could potentially be depleted by a separate effortful task. Participants underwent the rewarded Go/NoGo task, then a high or low load working memory task, and then the rewarded Go/NoGo reward task again. In the high load group, we observed a significant pre-to-post increase in the NoGo error rate for high reward trials ($p=0.02$), which was significantly greater than the pre-to-post change for low reward trials ($p=0.02$). This was not the case for the low load group ($p's>0.2$), most likely because top-down resources were not depleted. Taken together, these studies show that when a response is inappropriate, reward-predicting stimuli induce early motor activation, followed by a top-down effortful control process (which we interpret as response suppression) that depends on the strength of the preceding activation. Our findings provide novel information about the activation-suppression dynamics during control over rewarding stimuli, and they illustrate how fatigue or depletion leads to control failures in the face of reward.

Disclosures: **S. Freeman:** None. **D. Lu:** None. **A. Aron:** None.

Poster

080. Human Cognition: Control and Flexibility

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Program#/Poster#: 80.09/V44

Topic: F.01. Human Cognition and Behavior

Support: K12-DA031050

R01-DA020908

R01-DA035058

P50-DA09241

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P20-DA027844

Title: Sex differences in neural processing during fMRI monetary incentive delay performance

Authors: ***M. R. MITCHELL**[1], P. D. WORHUNSKY[1], I. M. BALODIS[1], M. C. STEVENS[2], G. D. PEARLSON[1], M. N. POTENZA[1];
[1]Psychiatry, Yale Univ. Sch. of Med., New Haven, CT; [2]Olin Neuropsychiatry Res. Center, Hartford Hosp., Hartford, CT

Abstract: Men and women differ in various psychopathological conditions, including addictions. For example, men are more likely to develop an addiction but women are more likely to telescope into an addiction and are also more likely to relapse. Sex differences in the neural mechanisms of various aspects of addiction (e.g., reward processing) have yet to be fully understood. In order to investigate sex differences in reward processing, we recruited healthy male and female participants to perform a monetary incentive delay task while undergoing functional magnetic resonance imaging (fMRI). Seventy-nine healthy male (n=41) and female (n=38) participants matched on age, race/ethnicity, and education completed a monetary incentive delay (MID) task during fMRI. The MID task consists of trials during which participants press a button in response to a target object in order to win, or avoid losing, monetary rewards of $0, $1, and $5. The MID task allows investigation of neural activity associated with different temporal phases of reward-processing, including reward-prospect,

reward-anticipation and reward-receipt. During win-anticipation women display greater ventral striatum (VS) and decreased posterior cingulate cortex (PCC) relative to males. In response to winning outcomes women exhibited reduced activation in the ventromedial prefrontal cortex (vmPFC), PCC, and anterior cingulate cortex. During loss-anticipation, women display greater activation in the cerebellum, PCC, mFC, inferior frontal gyrus (IFG), and VS. In response to a loss outcome, women displayed decreased activity in the dorsal striatum and increased activity in the middle temporal gyrus (MTG) than men. Sex-differences were also displayed during neutral trials, with women showing increased activity across reward-processing phases in the VS, IFG, vmPFC, and MTG. Taken together, these results suggest that men and women process rewards differently in that they have differential recruitment of brain regions during anticipatory and outcome phases. Importantly, sex differences were also observed during the neutral conditions (i.e., in the absence of a monetary incentive) with women exhibiting patterns of increased activity in reward-circuitry. Thus, men and women differ not only in their reward processing, also in their baseline activity during task performance. Such findings emphasize the need for a better understanding of the differences in neural processing between men and women with respect to various behavioral aspects of addiction such that better, and potentially sex-dependent, treatment options can be developed.

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Poster

080. Human Cognition: Control and Flexibility

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Program#/Poster#: 80.10/V45

Topic: F.01. Human Cognition and Behavior

Support: Pease Scholarship

College of Human Sciences Scholarship

Title: Prefrontal activation across time during executive tasks in young adults: A NIRS study

Authors: J. K. LANGE, *A. L. SMILEY-OYEN;
Kinesiology, Iowa State Univ., Ames, IA

Abstract: Introduction. We examined activation of the prefrontal cortex while performing two executive processing tasks using near-infrared spectroscopy (NIRS). We tested young adults to assess changes in underlying activation from task initiation to completion. While change across time has not been reported, fMRI studies indicate right lateralized activation during inhibition tasks and bilateral activation during tasks that require manipulation or updating of information (working memory). Methods. Changes in concentration of dorsolateral prefrontal blood oxygenation were assessed while 38 young adults (ages 18 to 25) completed the Stroop inhibition task and N-Back-1 and N-Back-2 working memory tasks. Results. Young adults exhibited right-lateralized prefrontal activation throughout the duration of the Stroop task. They exhibited increasing bilateral prefrontal activation during the N-Back 2 task. In addition, greater activation was elicited during the N-Back 2 task compared to the Stroop. Discussion. Prefrontal activation throughout the duration of executive task performance in young adults has not been previously studied. Task-related differences in activation appear to be the result of the cognitive effort necessary to perform executive tasks that require inhibition or working memory. Assessing the changes in activation throughout the course of executive tasks can provide new insights about distinct patterns of activation during executive function. These data provide a basis for future examination of changes in underlying activation in the prefrontal cortex that are age- and disease-related.

Disclosures: J.K. Lange: None. **A.L. Smiley-Oyen:** None.

Poster

080. Human Cognition: Control and Flexibility

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Topic: F.01. Human Cognition and Behavior

Support: Norsk Forskningsråd

Title: Orienting- and executive control deficits after exposure to life-threatening events

Authors: *C. S. SKAFTNES[1], A.-K. SOLBAKK[2,3], T. ENDESTAD[1];
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Abstract: Background: Several studies using tasks involving cognitive control (e.g., Stroop, Flanker, and Go/No-Go) have demonstrated significantly increased interference effects in posttraumatic stress disorder (PTSD) patients. However, it is still unclear whether the impairment is related to dysfunctions in proactive or reactive control. Moreover, as studies often use diagnosed PTSD as the main inclusion criterion, the observed alterations in performance may be driven by trauma exposure rather than being unique to PTSD. **Methods:** Using the Attention Network Task (ANT; Fan et al., 2002) we examined task performance in 28 adolescents who survived the terror attack in Norway (Utøya) on the 22nd of July 2010, compared to a non-trauma exposed control group (N = 22). Our main goal was to investigate whether the effects of valid versus invalid target location cues differed between groups, rather than target congruency. We predicted that an invalid cue would affect performance, measured by reaction times to a larger degree in the Utøya sample, compared to our control group. **Results:** The results revealed a main effect of both target congruency, $F = 212.20$, $p < .0001$, and cue validity, $F = 179.85$, $p < .0001$. Confirming our predictions, there was a significant interaction between cue validity and group, $F = 7.51$, $p = .009$, in that the trauma exposed group showed a significantly larger cue-dependent performance. However, the target congruency x group interaction was not significant, $F = .22$, $p = .644$. **Conclusion:** The results show that participants exposed to life-threatening events had more cognitive interference by an invalid cue compared to the non-exposed control group. Considering that the invalid cue was always centered, and could thus never be valid, our findings suggest that increased interference effect on cognitive control tasks may be a result of impaired proactive preparation effects by cues rather than lessened reactive control.

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Poster

080. Human Cognition: Control and Flexibility

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Topic: F.01. Human Cognition and Behavior

Support: cd ..

Title: fMRI activation of dorsal and ventral right inferior frontal cortex in a context-dependent stop signal task indicates different roles in motor control

Authors: *K. Z. XU[1], A. W. SALI[1], B. A. ANDERSON[1], S. YANTIS[1,2,3], S. M. COURTNEY[1,2,3];
[1]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]F.M. Kirby Ctr., Kennedy Krieger Inst., Baltimore, MD

Abstract: Human lesion and imaging (fMRI) studies have demonstrated that the right inferior frontal cortex (rIFC) is critical for stopping action, but the specific function of rIFC activity in stopping is debated. While some argue for a direct role of rIFC in response inhibition, others have argued for a role of rIFC in either guiding attention to external events, or in encoding behaviorally-relevant task rules. To test these hypotheses, we administered a context-dependent stop signal task to humans. Each participant received 3 types of trials: Go trials, Stop trials, and Continue trials, presented in a pseudo-randomized order. All trials started when participants acquired central fixation at a context cue. The shape of the context cue (square or triangle) indicated the stimulus-response rule mapping on that trial, and was visible briefly before it was replaced by a central fixation point. The fixation point was then extinguished and, simultaneously, a peripheral target appeared. On Go trials, participants were required to generate a speeded saccade to the target. On Stop and Continue trials, a yellow or blue point appeared at the center of the screen after a variable delay. In one context, a yellow point cued the participants to cancel the saccade while a blue point cued the participants to complete a saccade to the peripheral target. In the other context, the rules were reversed. The context alternated every 8,10 or 16 trials, randomly and without prior warning. Reaction times in Continue trials were longer than in the Go trials, and the Failed Stop trials yielded the shortest reaction times. Increased BOLD activity in dorsal rIFC was observed in both Stop and Continue trials compared to Go trials, but there was no activation magnitude difference between Stop and Continue trials. On the other hand, multivariate pattern analysis (MVPA) was able to classify, with above chance accuracy, the meaning of the instructional stimulus for that trial (i.e., Stop signals vs. Continue signals), but not the color of the stimulus (i.e., yellow vs. blue). These results suggest that dorsal rIFC 1) detects signals that are behaviorally relevant, and 2) represents the current stimulus-response association. In contrast, ventral rIFC demonstrated increased BOLD activity relative to Go trials, only in Stop trials, yet there was no activation magnitude difference between Failed and Successful Stop trials. Thus, contrary to some previous claims, these results suggest that the ventral rIFC detects signals that are stop-relevant, yet does not control motor inhibition per se.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: MOST Grant 103-2410-H-002-240-

Title: Distinct roles for left and right rostrolateral prefrontal cortex in information integration: A multivariate fMRI study

Authors: *T.-R. HUANG[1], R. C. O'REILLY[2];
[1]Dept. of Psychology, Natl. Taiwan Univ., Taipei, Taiwan; [2]Dept. of Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: The most anterior part of the brain is often referred as the rostral prefrontal cortex or frontal pole, which is primarily occupied by the Brodmann Area 10. In the literature of human executive control, the rostromedial prefrontal cortex (RMPFC) is implicated in mentalizing and multitasking, whereas the rostrolateral prefrontal cortex (RLPFC) is implicated in retrieval and integration of memory. For RLPFC, some laterality differences have been observed. For example, Bunge, Helskog, Wendelken (2009, NeuroImage) reported engagement of left, but not right, RLPFC in relational integration. However, the nature of such a laterality difference is not entirely clear from univariate analysis--left RLPFC may supervise rather than serve information integration, or vice versa. To examine the representations and functional roles of left and right RLPFC, we carried out a human functional magnetic resonance imaging (fMRI) study in that participants were challenged by a relational matching task, which is known to engage RLPFC. We then applied a multivariate pattern analysis (MVPA) to search for brain areas that encode task-specific information. Contrary to the results from univariate analysis in earlier studies, our preliminary data (N=6) from multivariate analysis show that right, but not left, RLPFC encodes both first-order and second-order relations, suggesting a direct contribution of right RLPFC to serve information integration. To provide further evidence, a more detailed and complete analysis (N=20) is being pursued.

Disclosures: T. Huang: None. **R.C. O'Reilly:** None.

Poster

080. Human Cognition: Control and Flexibility

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Program#/Poster#: 80.14/W1

Topic: F.01. Human Cognition and Behavior

Title: Tracking changes in EEG, mindful awareness, stress, and neuro-phenomena as personal perspective changes to universal perspective through Guided Subtraction Meditation

Authors: *G. K. SCHEINER[1], G. Z. SOGHOYAN[2], R. J. GOUGELET[2], A. M. VAHID[3], J. A. PINEDA[2];
[1]Cognitive Sci., UCSD, LA Jolla, CA; [2]Cognitive Sci., [3]Psychology, Univ. of California San Diego, La Jolla, CA

Abstract: We investigated the effects of the Guided Subtraction Meditation(GSM) practice, a new and unique meditation practice originating from South Korea. This practice focuses on dissociating from the egoic mind through a method they call subtraction. In this method, practitioners recall images and thoughts pertaining to past, present, and future experiences and discard or let go of them for the purpose of dissociating from these mental schema. Purportedly, after 100-150 hours of GSM, practitioners experience a state where the separation between the observer and observed dissipates, called the Universe Mind by the practice. We longitudinally tracked practitioners of this meditation(n=15) alongside non-meditating control subjects(n=20) from the start of their meditation practice to their 300-350th hour of meditation practice. At three timepoints(start, 100-150 hrs, 300-350 hrs or every 8-14 weeks for non-meditators), we administered the Philadelphia Mindfulness Inventory(PMI) and asked practitioners to fill out neurophenomenological reports(NPR). We also recorded EEG during 3 conditions, an eyes closed baseline, a session of GSM meditation, and a "stillness" task in which practitioners were asked to keep their mind free of thoughts. Between start and mid- treatment timepoints for the meditation group, we found significant trait increases in mindful awareness through the PMI and observed alterations in self experience related to ego dissolution and a feeling of universal oneness during the "stillness" task in practitioners' NPRs. The all electrode power spectrum showed significant increases in Theta, Alpha peak, and Beta peak power between start and mid-treatment timepoints across all conditions. While practicing GSM, Theta power decreased and Alpha power increased compared to baseline. These patterns are consistent with those seen during cognitive tasks that engage the DMN and increase cognitive load. This suggests that during GSM, the DMN may be engaged in a unique way while practitioners recall and actively dissociate from memories, thoughts, and feelings while simultaneously inhibiting mind-wandering and self-referential thought processes. During the "stillness" task, when practitioners reported experiencing the Universe Mind, Alpha spread over areas implicated with the ego body-self association in the TemporoParietal Junction, suggesting a potential neural basis for this change in self experience. Our observations suggest that this practice may be an effective method to induce rapid global changes in mental function which include increased awareness and alterations in self experience which seem to be safe and potentially healthy.

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Poster

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Title: The wandering brain: Individual differences in grey and white matter structure predict frequency of goal-related and emotionally positive mind-wandering

Authors: ***K. C. FOX**, M. S. JARRETT, M. GIRN, A. RAUSCHER, K. CHRISTOFF;
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Abstract: Mind-wandering (or 'spontaneous thought') has been most famously tied to activity in brain regions of the default mode network (DMN). However, almost nothing is known about how the brain's anatomical structure might vary in individuals with differing overall patterns of mind-wandering. For instance, individuals show marked differences in the frequency with which their spontaneous thoughts are goal-related (vs. unrelated) and emotionally positive (vs. neutral or negative) - but are these individual patterns of spontaneous thinking reflected at the level of neuroanatomy? We sought to explore the relationships between these individual propensities and both grey matter concentration (using high-resolution T1 anatomical MRI scans) and white matter integrity (using diffusion tensor imaging). During an MRI scan, we allowed subjects to rest and think freely, interrupting their thinking at random intervals with occasional thought probes. A total of 120 probes asked subjects about (i) whether their thoughts arose spontaneously, or whether they were intentionally directing them; (ii) whether thoughts were related to their current concerns and goals in life, or not; and (iii) whether they were emotionally pleasant, unpleasant or neutral. Overall individual difference scores were calculated for each participant (e.g., proportion of positive thoughts), and correlated with whole-brain grey matter concentration and tract-based white matter integrity. We found distinctive patterns of both grey and white matter structure correlated with individual propensity toward spontaneously arising vs. intentionally directed thoughts; thoughts related vs. unrelated to current concerns and goals; and emotionally pleasant vs. unpleasant thoughts. Moreover, these differences were observed in many regions beyond the DMN. Our results suggest that distinctive individual tendencies in the content and valence of spontaneous thinking are linked to correspondingly distinctive brain

anatomy. Our findings also speak to clinical disorders involving persistent and dysfunctional forms of spontaneous thought, e.g. depressive rumination: our results show that the brain structure of people who tend to have negative, non-goal-related thoughts is quantitatively distinct from those who tend to spontaneously produce positive, goal-related thinking.

Disclosures: K.C. Fox: None. **M.S. Jarrett:** None. **M. Girn:** None. **A. Rauscher:** None. **K. Christoff:** None.

Poster

080. Human Cognition: Control and Flexibility

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 80.16/W3

Topic: F.01. Human Cognition and Behavior

Title: Time course of conflict processing modulated by brief mindfulness meditation

Authors: *Y. TANG[1], R. TANG[2], M. POSNER[3];
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Abstract: Resolving conflict is a pivotal self-control ability for human adaptation and survival. Although some studies reported meditation may affect conflict resolution, however, these studies were not randomized and could not provide conclusion on causality. Moreover, the neural mechanisms of resolving conflict following meditation are poorly understood. Based on our series of studies using mindfulness meditation, we here conducted a fully randomized 5 hours trial of one form of mindfulness meditation - Integrative Body-Mind Training (IBMT) in comparison to a relaxation training control. Thirty-five healthy and right-handed undergraduates (mean age=21.31, 17 male) without any prior meditation or relaxation training experience participated in this study. The experiment was approved by a local Institutional Review Board, and informed consent was obtained from each participant. Before and after training, all subjects performed a Stroop word-color task while their brain activity was measured using a high-density electroencephalography system. Eighteen subjects had 10 consecutive IBMT sessions with about 30 min per day (5 h in total), seventeen subjects were given the same amount of relaxation training. These two training sessions were conducted in parallel. During the Stroop word-color task, IBMT group produced significantly faster resolution of conflict compared to relaxation group, a smaller N2 and an earlier and larger P3 component of the event-related brain potentials (ERPs). Source analysis using sLORETA showed that the P3 localized bilaterally to the dorsal part of the anterior cingulate cortex (ACC, BA 24/BA 32). These results indicate that brief

mindfulness meditation induces a brain state that modulates the activity of ACC and improves information processing including the resolution of Stroop conflict. These findings are compatible with the idea that IBMT improves cognitive flexibility and reduces habitual response via enhanced self-control. Acknowledgements This work was supported by the Office of Naval Research. We thank lab members for assistance with data collection and analysis. References Tang YY, et al. (2007). Short-term meditation training improves attention and self-regulation. Proc Natl Acad Sci U S A. 104, 17152-17156. Tang YY, Hölzel BK, Posner MI. (2015). The neuroscience of mindfulness meditation. Nat Rev Neurosci. 16, 213-25.

Disclosures: Y. Tang: None. **R. Tang:** None. **M. Posner:** None.

Poster

080. Human Cognition: Control and Flexibility

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Support: Canadian Institute of Health Research

Title: Is increased beta band power in the subthalamic nucleus related to global suppression of cortico-spinal excitability during behavioral response inhibition?

Authors: *A. GHAHREMANI[1,2], J. R. WESSEL[3], K. UDUPA[1], U. SAHA[1], M. HODAIE[1,2,4], A. M. LOZANO[1,4], S. K. KALIA[1,4], A. R. ARON[3], R. CHEN[1,5];
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Abstract: Behavioral response inhibition is often studied with the stop-signal task, in which participants prepare a quick response to a go-stimulus, and then sometimes try to stop the initiated movement on trials in which a stop-signal occurs. Several studies in humans show that rapid behavioral response inhibition has global effects on the skeletomotor system. For example, when participants stop, corticospinal excitability (CSE), assessed with single pulse Transcranial Magnetic Stimulation (TMS), is reduced not only in the target muscle that needs to be stopped, but also in task-unrelated muscles as well. Rapid stopping recruits the subthalamic nucleus (STN) of the basal ganglia, one signature of which is increased beta band power of local field potentials in the STN of Parkinson's disease patients. Given also the suggestion that the STN has a broad effect on basal ganglia output, we hypothesized that broad skeletomotor suppression

during stopping (as assessed with TMS) could relate to the increase of beta band power in the STN. To test this, we measured STN local field potentials from deep brain stimulation electrodes implanted in six Parkinson's disease patients. Subjects performed a verbal stop-signal task, while their CSE was concurrently measured with TMS pulses to the motor cortex and EMG from their right hand (which was task-unrelated). Behavioral stop signal performance was good. The mean Go RT was 708ms and the speed of stopping (SSRT) was 361ms. The probability of stopping was .54. In line with several reports (Ray et al. NeuroImage 2012; Alegre et al. Exp.Neurol 2013), there was increased STN beta-band activity for successful stop trials compared to failed stop trials and go trials ($F(2/10) = 5.18$, $p < 0.03$). Also, in line with several studies in healthy young participants (Wessel et al. J Neurophys 2013; Cai et al. Brain and Language 2012, Badry et al., J Neurophys 2009), CSE was reduced from the task-unrelated hand for successful stop trials compared to failed stop trials and go trials ($F(2/10) = 5.94$, $p < 0.02$). Preliminary analysis showed that trials with stronger STN beta-band activity had numerically stronger CSE suppression measured at the hand, but this did not reach statistical significance in this incomplete sample. Thus, consistent with prior reports, rapid action stopping increases beta band power in the STN and produces global skeletomotor suppression. Preliminary evidence suggests these may be related, providing some evidence for the idea that global motor suppression is mediated via the STN.

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Poster

080. Human Cognition: Control and Flexibility

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Topic: F.01. Human Cognition and Behavior

Support: FAPESP

CAPES

CNPq

AFIP

Title: Effects of socioeconomic status on executive impairment in young smokers

Authors: R. L. ANTONIO[1], *S. POMPEIA[2];
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Abstract: Cigarette smoking elicits deleterious effects on cognition and is the leading cause of preventable death worldwide. Eighty percent of the one billion smokers worldwide come from developing countries, with high rates of income inequality. Low socioeconomic status (SES) is not only associated with higher rates of smoking as with poorer cognitive stimulation, which negatively affects cognition including executive functioning, also reported to be impaired in smokers. However, details are lacking regarding which type of executive functions are affected by SES and smoking. This is so because the term executive function encompasses many domains related to control of behavior, which can be evaluated by tasks that assess inhibition, shifting, updating, planning, access to long term memory, dual tasking (cold executive domains) as well as impulsivity (hot executive function, which can be measured by tests or questionnaires), related to poorer inhibitory control and higher risk taking. Here we studied whether SES would mediate executive impairment in young smokers. In an ongoing study, 60 young (aged 18-35), healthy smokers (> 10 cigarettes a day for 1 year) and nonsmokers of high and low SES were assessed regarding cold executive functions considering the above mentioned domains (Stroop Test, Category Switch Test, Number Memory Test, BADS Zoo Map, verbal fluency, dual task), impulsivity (Barratt Impulsivity Scale, BIS-11; delay discounting) and control measures sensitive to SES (working memory capacity measured by the Counting Span Test). Effects of acute nicotine administration and withdrawal were controlled through a test battery that lasted 90 min and by having smokers carrying out the tasks 30 min after the last cigarette (less than two hours of abstinence). Testing sessions were carried out in the morning. We found that irrespective of smoking status low SES participants had worse scores in the control measures of working memory capacity, confirming the deleterious effect of lower cognitive stimulation. Inhibition, shifting, updating and access to long term memory were also impaired in the low SES participants in relation to the high SES group. Regarding hot executive functions, lower SES participants also reported worse control of impulsivity in the BIS-11 and higher delay discounting. Negative effects of smoking were only found in the BIS-11 (motor impulsivity, non-planning, and BIS total score) and it did not interact with SES. Our preliminary findings suggest that low SES impairs many cold and hot executive abilities and high SES does not protect smokers against the negative effects of tobacco use on impulsivity.

Disclosures: R.L. Antonio: None. **S. Pompeia:** None.

Poster

080. Human Cognition: Control and Flexibility

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 80.19/W6

Topic: F.01. Human Cognition and Behavior

Title: Cognitive control modulates task representations in occipital and prefrontal cortex

Authors: *L. S. LOOSE[1,2,5], D. WISNIEWSKI[3,2,5,6], M. RUSCONI[1], J.-D. HAYNES[1,2,6,4,7]; [1]Bernstein Ctr. for Computat. Neurosci., Charité Universtitätsmedizin Berlin, Berlin, Germany; [2]Berlin Ctr. for Advanced Neuroimaging, [3]Bernstein Ctr. for Computat. Neurosci., [4]Excellence Cluster NeuroCure, Charité - Universitätsmedizin, Berlin, Germany; [5]SFB 940 Volition and Cognitive Control, Technische Unviersität Dresden, Dresden, Germany; [6]Berlin Sch. of Mind and Brain, Humbolt Univ. zu Berlin, Berlin, Germany; [7]Dept. of Psychology, Humboldt Univ. zu Berlin, Berlin, Germany

Abstract: Alternating between different tasks is effortful and requires the recruitment of executive control functions. On trials following a task switch, as compared to task-repeat trials, reaction times are slower, which is known as a switch cost. Previous research on task preparation has compared global signal differences between switch and repeat trials. These studies often report a prefrontal network to be more active in switch-conditions than in stay-conditions. Recently, it has been shown that task sets are encoded in spatially distributed patterns of brain activity. So far, however, it has remained unclear whether these neural task representations are modulated by the increased control demands in switch trials. In this experiment, we used multivoxel pattern analysis (MVPA) in order to to compare the task-related neural information that is decodable in switch versus repeat trials. 40 subjects performed an event-related task switching paradigm, while functional magnetic resonance imaging (fMRI) was acquired. On each trial, subjects were cued to perform one of two different tasks. Trials either repeated the previous task or switched to the other task. Tasks were cued by abstract visual symbols presented on screen. The design allowed us to orthogonalize switch and repeat trials for both tasks. We used multivariate searchlight decoding to identify task-related processes information separately for switch and repeat conditions. To control for the influence of visual cue-related information, MVPA-classifiers were trained on data from one cue and tested on data from the other cue (cross-classification). In order to avoid overfitting, we applied leave-one-run-out cross-validation. We found that tasks could be decoded with higher accuracies in switch as compared to stay trials. In switch trials, task information was found in occipital lobe and the inferior frontal gyrus, pars triangularis, whereas analyses of repeat-trials yielded no regions with significant task-information. These results indicate that cognitive control seems to strengthen task representations under conditions of switching, primarily in occipital lobe and prefrontal cortex.

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Poster

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Support: F32 EY023922

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R01-EY01988

P30-EY08126

P30-HD015052

the E.Bronson Ingram Chair in Neuroscience

Title: Dissociable electrophysiological correlates of proactive and reactive control during response inhibition

Authors: *K. FUKUDA, J. D. SCHALL, G. F. WOODMAN;
Psychological Sci., Vanderbilt Univ., Nashville, TN

Abstract: The countermanding paradigm has been extensively used to examine our ability to inhibit a planned action. Theories propose that response inhibition is accomplished by two types of executive control. Proactive control uses endogenous expectations to inhibit actions, whereas, reactive control is exerted to fully terminate actions in response to the external cues (e.g., Verbruggen & Logan, 2009; Aron, 2011; Bisset & Logan, 2011; van Belle, et al. 2014). We recorded scalp EEG while human participants performed a manual countermanding task. We found that proactive and reactive control were instantiated by two distinct neural responses observed at different stages of the task. Proactive control occurred with a larger amplitude sustained negativity observed across frontal channels prior to target presentation. Reactive control occurred with a transient increase in alpha power observed at parieto-central channels following the stop signals. In Experiment 2, we found that these two mechanisms of executive control interact. Using pre-trial cues to indicate the probability of an upcoming stop signal modulated the sustained frontal negativity of proactive control. However, responses were successfully inhibited when the parieto-central alpha power of reactive control compensated for the level of proactive control following the cue. Our findings reveal novel neural metrics of

proactive and reactive control with high temporal precision and demonstrate the interaction of these mechanisms as subjects control responses.

Disclosures: **K. Fukuda:** None. **J.D. Schall:** None. **G.F. Woodman:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: FT120100033

SR120300015

Title: Training divides neural representations to conquer multitasking costs

Authors: ***K. G. GARNER**, P. E. DUX;
Sch. of Psychology, Univ. of Queensland, St. Lucia, Australia

Abstract: The present "Information Age" is replete with complexity and frequently requires that we manage multiple tasks concurrently. It is thought that humans are poor at multitasking because frontoparietal and subcortical (FP-SC) brain regions both serve a broad range of mental functions and are limited information processors. Thus, performing multiple tasks exceeds the capability of the system, and performance impairments are incurred. Training is known to improve multitasking ability by influencing the activity of FP-SC areas. However, it is not well understood how the functional organisation of this system changes to improve multitasking performance. To address this, we characterized the functional changes of the FP-SC system that predict multitasking-training outcomes. Participants (N=100) performed single and multiple tasks in pre- and post-magnetic resonance imaging (MRI) sessions that were interspersed by either a multitasking or an active-control training regimen (inefficient visual-search task). Using functional imaging and a multivoxel pattern analysis (MVPA) approach, we found that the divergence of FP-SC blood-oxygen-level-dependent (BOLD) response patterns across voxels to the trained tasks predicted multitasking improvements. Importantly, this was only observed for participants who trained on the multitasking regimen, and not for the active-control group. Therefore, the FP-SC system supports enhanced multitasking behavior by segregating representations for the constituent tasks, thereby reducing neural competition between them.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH AG025667

Title: Greater distribution of executive control networks supports cognitive reserve in bilingual older adults

Authors: ***T. B. WENG**[1], E. GUZMÁN-VÉLEZ[1], G. COOKE[2], A. Z. BURZYNSKA[2], C. N. WONG[2], E. MCAULEY[2], A. F. KRAMER[2], D. TRANEL[1], M. W. VOSS[1];
[1]The Univ. of Iowa, Iowa City, IA; [2]Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: A wealth of research supports the notion that bilingualism protects the aging brain by attenuating the cognitive declines that accompany healthy aging and by delaying the onset of dementia. These findings are consistent with the cognitive reserve hypothesis, which postulates that there are individual differences in the ability to adaptively cope with the decline in brain integrity and maintain cognitive functioning. How might bilingualism enable such cognitive reserve? One view posits that a lifetime of managing two languages produces flexibility between multiple brain networks, enabling a larger repertoire of paths for cognitive processing. Accordingly, we predicted that brain regions in the executive control network (ECN) of bilingual older adults are more functionally connected to regions from other cognitive networks when compared to their monolingual peers. In the current study, we tested this prediction by analyzing resting-state functional magnetic resonance imaging (rs-MRI) data from healthy bilingual (n = 10) and monolingual (n = 10) older adults matched in their age, years of education, and sex. With high spatial precision and test-retest reliability rs-fMRI measures functional connectivity, as expressed by temporal correlations of brain activity during the resting state, among multiple brain regions without the constraints of specific task demands. Thus, rs-fMRI permits one to observe the organization of spatially distributed brain regions into functionally connected networks. Consistent with our predictions, we found that, compared to monolinguals, the ECN of bilinguals was more functionally connected to the salience network (SAL), a network proposed for detecting behaviorally-relevant stimuli, and to the dorsal attention network (DAN), a network proposed for driving goal-driven attention ($p < 0.05$). However, we observed that a network comprised of classical language regions is more functionally differentiated from other networks (default network and ECN) for bilinguals compared to monolinguals ($p < 0.05$), suggesting greater functional specialization of language regions in bilinguals. We interpret these results as

reflecting a possible neural mechanism underlying the extended cognitive reserve found in bilingual older adults. We suggest that bilingualism is associated with greater integration of networks involved in executive control and attention accompanied by greater specialization of regions involved in language.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: BP-DGR Grant 2009 BP-A 00025

Title: Subcortical response increase to uncertainty and deviations from expectation

Authors: *A. MESTRES-MISSE[1], R. TRAMPEL[2], R. TURNER[2], S. A. KOTZ[1,2];
[1]Univ. of Manchester, Manchester, United Kingdom; [2]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: Optimal behavior often requires a precise balance between automatic and controlled processing. In a dynamic noisy environment, it is not only beneficial to automatize routinely reliable behavior, but also to allow flexibility to deal with uncertainty and unexpected events. The present 7T-fMRI study investigated the role of subcortical structures in regulating this automatic-controlled processing mechanism, especially necessary when our expectations are violated. For this purpose, a new task was designed, in which participants learned to calculate the outcome of different sequences of geometrical figures. The design included three types of sequences, which provide unambiguous, ambiguous and incongruent contextual information, and two outcomes, probable and unlikely. After three consecutive days of training in calculating the value of the sequences, participants judged the correctness of the sequences in the scanner. We hypothesize that when there is a difference between the expected and actual outcomes, cognitive control mechanisms are needed to override the anticipated response, re-evaluate the evidence, and select an appropriate, albeit less likely, alternative (if possible). The results showed increased dorsomedial striatal and ventral anterior thalamic nucleus activation for ambiguous and unambiguous subordinate sequences; additionally, ambiguous sequences displayed also larger

activation in bilateral centromedian-parafascicular thalamic nucleus and red nucleus; and finally, dorsolateral and posterior dorsomedial striatum showed increased activation for incongruent sequences. These results demonstrate how different subcortical structures modulate deviations from expectation as well as highlight their critical function in cognitive control and the automatic-controlled processing balance.

Disclosures: A. Mestres-Misse: None. **R. Trampel:** None. **R. Turner:** None. **S.A. Kotz:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: Cognitive control by disinhibition: a cortical model of decision making and multiple-item working memory

Authors: *D. STANDAGE, M. PARE, G. BLOHM;
Queen's Univ., Kingston, ON, Canada

Abstract: Recent data provide evidence for the control of cortical processing by interneuron-targeting interneurons (Pi et al, Nature, 2013). We hypothesize that such disinhibition is a fundamental mechanism of cognitive control, testing our hypothesis with a biophysically-based model of a local circuit in association cortex. It is widely believed that several association cortical areas (e.g. dorsolateral prefrontal cortex and the lateral intraparietal area) play an important role in decision making and working memory, but these two cognitive processes make conflicting demands of neural circuitry. Competition between neural populations encoding choice alternatives is crucial to decision making, but competition between populations encoding memoranda entails forgetting. We propose that disinhibition modulates the competition between neural populations, allowing the same circuitry to support cognitive tasks with different processing requirements. Our local-circuit model is a network of simulated pyramidal neurons and inhibitory interneurons, connected by AMPA, NMDA and GABA synapses. All biophysical parameters are justified by electrophysiological data, including the structure of synaptic connectivity, the relative strengths of synaptic conductance, and the high-conductance state of neurons due to background synaptic bombardment. We simulate disinhibitory control by modulating the strength of the inhibitory background conductance onto interneurons. In simulations of visual discrimination tasks, weak disinhibition reproduces signature

characteristics of neural and behavioural data, including the time-evolution of decision-correlated neural activity, and psychometric and chronometric curves. Disinhibition also controls the speed-accuracy trade-off, where a weaker disinhibitory signal produces slower, more accurate decisions for a given task difficulty and number of alternatives. In simulations of visual working memory tasks, strong disinhibition supports working memory capacity consistent with that of experimental subjects, qualitatively reproducing neural data from association cortical areas. Our disinhibitory signal further controls network performance in a third task, in which multiple stimuli are presented simultaneously, but none are chosen. Many decision making and working memory tasks include an interval of this description. Overall, our model provides compelling evidence for disinhibition as a source of cognitive control, accounting for a large volume of neural and behavioural data under a single set of parameter values, and making predictions for future experimental studies of cognition.

Disclosures: **D. Standage:** None. **M. Pare:** None. **G. Blohm:** None.

Poster

080. Human Cognition: Control and Flexibility

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Topic: F.01. Human Cognition and Behavior

Support: NSERC Canada

Title: Examining changes in cortical activity associated with switching attention between tasks of different modalities

Authors: *J. L. TOMLIN, W. E. MCILROY;
Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: With modern technology advancing, humans are constantly trying to perform multiple tasks at the same time. Such multitasking commonly involves switching attention between two tasks. This ability to disengage from one task and engage in another is an executive function allowing for cognitive flexibility in a dynamic environment. Attention switching can, however, result in decreased level of performance as measured as a behavioural switch cost. This switch cost is the difference in reaction times between trials involving a task switch and trials in which a single task is performed (no switch). The current study is focussed on advancing an approach to reveal the temporal properties of this attention switching and the underlying electrophysiological correlates. The rationale for this study was a precursor prior to evaluating exercise-induced

changes on the temporal characteristics and electrophysiological events associated with attention switching. In the current study, participants switched from a near-continuous background auditory task to a primary visual simple reaction time task. The background auditory task involved a choice reaction using the left hand based on the frequency of two auditory tones. The switch task was a simple visual reaction time task involving a right wrist flexion response to a left arrow. This background task was chosen so participants were continuously engaged in a task (with a 600 ms interval between auditory tones) and then must rapidly switch to the visual task at an unpredictable time point. The timing of the visual reaction times during switch and non-switch trials were compared to determine switch cost. Continuous EEG was examined while switching between these tasks as well as during the performance of the visual task repetitively. ERPs time-locked to the visual stimulus were examined. Preliminary results revealed a delayed P3 latency in the switch condition, indicating the stimulus evaluation stage of information processing is longer when switching attention between two tasks. Further analysis will investigate earlier ERP components to examine cortical activity differences in early stages of information processing comparing switch and non-switch trials. This research hopes to reveal which stages of information processing are altered in timing when switching attention between two tasks and the factors that modulate capacity for attention switching.

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Poster

080. Human Cognition: Control and Flexibility

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Topic: F.01. Human Cognition and Behavior

Support: Abbott Center for Nutrition Learning and Memory Grand Challenge

NIMH (MH63901)

Title: Effects of tolcapone and bromocriptine on cognitive flexibility in an anti-saccade task

Authors: *I. G. CAMERON[1], D. WALLACE[2], A. AL-ZUGHOUL[3], A. S. KAYSER[2], M. D'ESPOSITO[3];
[1]Ctr. for Cognitive Neuroimaging, Donders Inst. for Brain, Cognition and Behavio, Nijmegen, Netherlands; [2]Univ. of California, San Francisco, San Francisco, CA; [3]Univ. of California, Berkeley, Berkeley, CA

Abstract: In terms of executive functions, it has been suggested that the prefrontal cortex (PFC) is important to task maintenance, but that the basal ganglia (BG) are important to task flexibility. Tolcapone and bromocriptine are dopaminergic drugs that can be used to investigate such assumptions. Tolcapone, a COMT inhibitor, should affect behaviors that rely on PFC function, because by inhibition of COMT, more dopamine remains at the synapse (COMT has greater importance for PFC compared to striatal dopamine metabolism). In contrast, bromocriptine, at D2 agonist, should affect behaviors, such as task switching, that more explicitly recruit PFC-BG circuits, due to the relative dominance of D2 receptors in the BG than in the PFC. We used a pro- (look towards) /anti- (look away) saccade task that included the requirement to switch suddenly to the opposite task mid-trial. Our hypothesis was that task maintenance depends on the PFC; therefore, tolcapone would improve performance on non-switch trials, however it would impair performance on switch trials. Conversely, bromocriptine would facilitate switch trials, but would impair non-switch trials. 16 subjects participated in a 3-session double-blind experiment counterbalanced for tolcapone, bromocriptine or placebo. 40 % of the trials were non-switch trials and 60 % of the trials were switch trials. Target stimuli were presented pseudorandomly to the left or right. A measure of "efficiency" (percent correct / reaction time) was used to assess overall performance. We did not find a significant Drug X Switch Condition interaction ($P = 0.06$), though this trend indicated that non-switch trials were less efficient under tolcapone. Tolcapone reduced efficiency overall (main effect of Drug, $P < 0.01$), and efficiency under tolcapone was different from placebo ($P < 0.05$). To further explore this, we divided subjects based on COMT Val/Met polymorphism (Val results in a more active COMT enzyme), to determine if there was a Drug X Genotype interaction. This interaction was not significant ($P = 0.26$), but there was main effect of genotype ($P < 0.05$), such that Val/Val subjects were the most efficient and there was a Genotype X Switch Condition interaction ($P < 0.01$), as Val/Val subjects also exhibited less of a loss in efficiency on switch relative to non-switch trials. We suspect that the nature of the task (2 Tasks, 2 Switch Conditions and 2 Target Locations) requires trial-by-trial flexibility. Thus, optimal performance necessitates subjects to be governed by the task stimuli: programming an initial response, and switching if, and only if, instructed explicitly. The results suggest that greater PFC dopamine impedes cognitive flexibility.

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Poster

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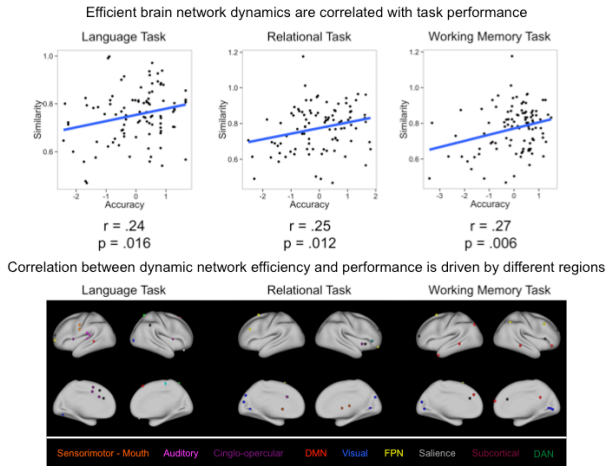
Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH096801

Title: Efficiency of brain network dynamics associated with cognitive ability

Authors: *D. H. SCHULTZ, M. W. COLE;
CMBN, Rutgers-Newark, Newark, NJ

Abstract: The efficiency of the human brain is remarkable. It is able to exceed modern computers on multiple computational demands (e.g., language, planning) while consuming the wattage of a mere light bulb. The mystery of how the brain can be so efficient is compounded by recent evidence that all brain regions are constantly active as they interact with each other in so-called resting-state networks (RSNs). In order to investigate the brain's ability to efficiently process complex cognitive demands we used data from the Human Connectome Project (WU-Minn consortium, N=100) to compare functional connectivity during rest and several highly distinct tasks (as previously described; (Barch et al., 2013)). We previously found that RSNs are present during a wide variety of tasks, and that tasks only minimally modify functional connectivity patterns throughout the brain (Cole et al., 2014). Here we tested the hypothesis that, while subtle, these task-evoked functional connectivity changes from rest nonetheless strongly contribute to behavioral performance. Surprisingly, we found across three diverse tasks (language, relational, and working memory tasks) that high performing individuals exhibited more efficient brain connectivity updates (smaller changes in functional network architecture between rest and task). These results suggest that individuals with more efficient brain connectivity updates have especially effective network configurations for general task performance. The brain's efficiency therefore appears to be a key feature contributing to both its network dynamics and general cognitive ability.



Disclosures: D.H. Schultz: None. **M.W. Cole:** None.

Poster

080. Human Cognition: Control and Flexibility

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 80.28/W15

Topic: F.01. Human Cognition and Behavior

Title: Executive functions of children within a social street context in the downtown area of guadalajara

Authors: C. A. CASTAÑEDA NAVARRETE[1], T. VILLASEÑOR CABRERA[2], M. JIMÉNEZ MALDONADO[2], A. JARNE ESPARCIA[3];
[2]Neurosci., [1]Univ. of Guadalajara, Guadalajara, Mexico; [3]Dept. of Personality, Evaluation and Psychological Treatments, Univ. of Barcelona, Barcelona, Spain

Abstract: Introduction: Early experiences are critical for the Brain development in Children, and maturation of their cortical areas. When children are exposed to different kinds of threaten, for example work in the street on an average of 6 or 7 hours per day, plus the lack of education, an unhealthy environment, due a low socioeconomic status (SES). These children suffer an affectation on their Neurocognitive functions development, which will bring serious consequences in their future. **Objective:** The aim of this work is to compare the performance of executive functions and cognitive process in children aged from 7 to 11 years old, considering the SES, gender, as well the school status. We looked to describe the Neuropsychological profile of children in a street social context. **Methods:** Participants were 40 children from 7 to 11 years old, based on the downtown of Guadalajara city, were divide in two groups, 20 inside of a street context, and 20 from a structure family context with a medium SES. We used the Neuropsychological Questionnaire school maturity (CUMANES) this questionnaire have 13 sub tests, and 8 sub tests from the neuropsychological battery of executive functions (BANFE), which evaluate different neuroanatomical areas of the prefrontal cortex. **Results and Discussion:** We completed a sample of 20 Children in groups, 10 girls and 10 boys, the group from a street context presented a low Neuropsychological development index compared with the other group, they showed problems in planning abilities, working memory, and semantic tasks. Although a few subjects of street context group present a different range but maybe is for ethnic characteristics. The lack of Neuropsychological skills increased, with the differences age in the street context group, presented alterations in different neuropsychological domains**.**

Disclosures: T. Villaseñor Cabrera: None. **M. Jiménez Maldonado:** None. **A. Jarne Esparcia:** None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.01/W16

Topic: F.01. Human Cognition and Behavior

Support: McDonnell Foundation

Title: Cognitive effort and the opportunity cost of time: a behavioral examination

Authors: *R. OTTO, N. DAW;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: A spate of recent work demonstrates that humans seek to avoid the expenditure of cognitive effort, much like physical effort or economic resources. Less is clear, however, about the circumstances dictating how and when people decide to expend cognitive effort. Here we adopt a popular theory of opportunity costs and response vigor and to elucidate this question. This account, grounded in Reinforcement Learning, formalizes a trade-off between two costs: the harder work assumed necessary to emit faster actions and the opportunity cost inherent in acting more slowly (i.e., the delay that results to the next reward and subsequent rewards). Recent work reveals that the opportunity cost of time--operationalized as the average reward rate per unit time, theorized to be signaled by tonic dopamine levels, modulates the speed with which a person responds in a simple discrimination tasks. We extend this framework to cognitive effort using established “cognitive control” tasks, for which 1) the amount of cognitive effort demanded from the task varies from trial to trial and 2) expenditure of cognitive effort holds consequences in terms of accuracy. In one experiment, 50 subjects completed a Simon task while available rewards varied randomly on a trial-to-trial basis. Echoing previous results, we found that subjects tuned their response speeds in accordance with the experienced average reward rate: when the opportunity cost of time was high, subjects responded more quickly. On response-incongruent trials--for which correct responses demand cognitive effort--this reward-rate-dependent speeding was accompanied by a reduction in accuracy. That is, expenditure of cognitive effort appeared to be modulated by the opportunity cost of time. Further, and consistent with our account, the strength of this modulation covaried with individual differences in efficacy of cognitive control, operationalized as response slowing on incongruent trials. Taken together, our results provide an initial look the circumstances dictating how and when people expend cognitive effort.

Disclosures: **R. Otto:** None. **N. Daw:** None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.02/W17

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant K12HD073945

Title: Measuring the subjective cost of physical effort

Authors: *P. S. HOGAN[1], C. D. FRYDMAN[2], V. S. CHIB[1,3];
[1]Biomed. Engin., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]USC Marshall Sch. of Business, Los Angeles, CA; [3]Kennedy Krieger Inst., Baltimore, MD

Abstract: We constantly make decisions about what types and how much effort we exert. One factor that could influence these decisions is our subjective preferences for effort (i.e. how effortful a task feels). In this experiment we characterized individuals' subjective preferences for physical effort costs and their underlying neural mechanisms. To accomplish this we scanned subjects with fMRI while they performed a physical effort task that required exertion in the form of gripping a hand-clench dynamometer. Subjects first learned an association between force produced and a numerical scale ranging from zero to one-hundred relative to subjects' maximum voluntary contraction. They then performed a series of risky binary gambles involving varying levels of force to be exerted. During each choice subjects made a decision between exerting a low amount of effort with certainty or a gamble that could result in a high level of exertion or a zero level of exertion. We found that the majority of subjects exhibited convex subjective effort cost functions, indicating that the marginal subjective values of the cost associated with the task increased as more effort was required. A total of 20 subjects have been scanned and subsequent analysis will look for brain regions with activity associated with individuals' subjective preferences for physical effort.

Disclosures: P.S. Hogan: None. **C.D. Frydman:** None. **V.S. Chib:** None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.03/W18

Topic: F.01. Human Cognition and Behavior

Support: Fundacion Obra Social La Caixa

Institute for Culture and Society (ICS)

Title: The influence of habitual physical exercise on effort discounting in decision making

Authors: *J. BERNACER[1,2], E. LUIS[3], I. MARTINEZ-VALBUENA[2], M. MARTINEZ[3], N. PUJOL[4], D. RAMIREZ-CASTILLO[2], M. A. PASTOR[3,2];
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Abstract: Effort-based discounting on decision making has been demonstrated to correlate with sedentary behavior. In the present contribution, we hypothesize that changing sedentary lifestyle for habitual physical exercise may reduce effort-based devaluation, resulting also in functional brain changes during decision making. To test this hypothesis, we recruited 22 young participants (age 18-24) with a sedentary behavior according to the GPAQ test (World Health Organization), and included them in a 3-month mild physical activity program (3 days a week, 30 minutes per day). They completed several neuropsychological tests (RYFF, TCI) and were tested using BOLD fMRI before and after the program while performing a decision-making task as follows: subjects were presented pairs of options that differed in probability (odds to win) and effort (minutes running on a treadmill) to earn a fixed payment (30 euros), so the volunteers had to balance high probabilities of winning with their willingness to run. They were instructed that one of the pairs of options (out of a total of 315) would be randomly selected by a lottery and they had to perform the selected amount of exercise in order to get the money. The neurocomputational analysis of fMRI data revealed that, before following the physical training plan, two clusters located in the dorsal anterior cingulate cortex and dorsomedial prefrontal cortex positively correlated with uncertainty (computed as Shannon entropy) and subjective value, respectively (both $P<0.05$, FWE corrected for multiple comparisons at cluster level). After completing the program, subjects tended to choose the high probability option of the pair irrespective to the physical effort (paired t-test before vs after the program, $P<0.05$). We also found robust changes in the fMRI coding of uncertainty and effort discounting (voxel-by-voxel paired t test): 1) uncertainty values (Shannon entropy) showed a significant positive correlation with BOLD signal in the precuneus after the program, compared with before the program ($P<0.05$, FWE corrected for multiple comparisons at cluster level); 2) effort-discounting values significantly positively correlated with BOLD signal in the ventromedial prefrontal cortex after the program, compared with before the program ($P<0.05$, FWE corrected for multiple comparisons at cluster level). In all, our experiment confirms that habitual physical exercise done

by formerly sedentary subjects impacts on effort-based decision-making both at behavioral and neural levels.

Disclosures: **J. Bernacer:** None. **E. Luis:** None. **I. Martinez-Valbuena:** None. **M. Martinez:** None. **N. Pujol:** None. **D. Ramirez-Castillo:** None. **M.A. Pastor:** None.

Poster

081. Value-Based Human Decision Making

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.04/W19

Topic: F.01. Human Cognition and Behavior

Support: The Wellcome Trust

Title: Option generation and option switching decision-making in apathy - a disorder of motivation

Authors: ***Y. ANG**, M. HUSAIN;
Univ. of Oxford, Oxford, United Kingdom

Abstract: Apathy or lack of motivation to act is a profoundly disabling clinical condition that also occurs in milder form in healthy people. The mechanisms underlying its manifestations are largely unknown, but it has been suggested that it might arise from deficits in decision making. Here we examined the relationship between apathy and (i) option generation, and (ii) option switching in healthy young and older adults. We used two novel paradigms implemented on a touchscreen computer while level of motivation in participants was indexed using a modified version of the Lille Apathy Rating Scale. In the first task, participants (young: N = 40; elderly: N = 25) were required to produce, in four minutes, as many different paths as possible between a start-point and an end-goal. Three conditions were tested, with different levels of effort required (0, 4 or 8 barriers). In each condition, the number of paths generated correlated strongly with motivation, independent of age, baseline motor ability and IQ. In the second task, participants (young: N = 25; elderly: N = 20) had three minutes to maximise rewards earned by producing as many paths as they could, but now they had a choice between two goals. Each path was drawn from a start-point to either a (i) high reward goal that became progressive harder to reach with new barriers appearing each time a path was drawn to it, or (ii) low reward goal with no barriers. Compared to motivated people, apathetic individuals actually allocated more effort during the task. They persevered longer on going to the high-reward goal (which progressively became harder to reach), switching to the easier, low-reward option much later than more motivated

participants. Yet, as a result apathetic people earned less credits. Using each individual's movement time to reach the low-reward goal, we also computed the optimal number of paths to the high-reward goal before a participant should switch to the low-reward goal. Deviation from the optimal strategy scaled negatively with motivation level, i.e. apathetic people were less able to initiate effective responses, deviating more from the optimal strategy. These findings demonstrate that lack of motivation is associated with both impaired option generation and option switching goal-directed behaviour. Apathetic people were not only slower at generating options, but were also less able to switch effectively between them. These simple tasks serve as tools to probe disrupted decision-making mechanisms underlying motivational deficits, providing objective methods for examining pathological apathy in brain disorders.

Disclosures: **Y. Ang:** None. **M. Husain:** None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.05/W20

Topic: F.01. Human Cognition and Behavior

Support: ERC Advanced Grant 293549

Title: Neural processes underlying reward-saving behavior in human amygdala and prefrontal cortex

Authors: *L. ZANGEMEISTER, F. GRABENHORST, W. SCHULTZ;
Dept. of Physiology, Develop. and Neurosci., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Saving behavior has a profound influence on markets and entire economies. Contrary to common binary inter-temporal choice paradigms, deliberate saving requires internal planning and repeated postponement of consumption across time. We recently showed that, during reward-saving, single neurons in the monkey amygdala exhibit planning activity related to single-step economic choices (Grabenhorst et al., 2012) and longer-term internal reward goals (Hernadi et al., 2015). We designed a saving paradigm and used fMRI to explore the neural mechanisms underlying internally planned reward-saving in humans. Healthy volunteers (N = 24) freely set reward goals and subsequently performed choice sequences of up to three minutes to attain primary rewards (milk-based drinks). To elicit variation in saving behavior we used different reward types (high/low fat content) and rates of reward increase during saving (high/low growth). Reward type and growth rate were cued at the start of each choice sequence

allowing subjects to form an internal saving plan. Before sequence initiation, subjects reported their willingness-to-save. During a sequence, subjects chose trial-by-trial to save the reward for later or to spend (i.e. consume) immediately. Individual saving behavior reflected subjective valuations of reward type, growth rate and their interaction. Subjects did not always follow their initially reported plan, which allowed us to distinguish neural activity related to stated intentions from activity predictive of the eventually executed saving sequence. Analysis of fMRI data showed that amygdala activity reflected both subjects' internal saving plans and trial-by-trial choices ($P < 0.05$, cluster-level corrected). At sequence start, amygdala planning activity more strongly encoded the future executed saving sequence than reported willingness-to-save. During sequence execution, amygdala activity encoded trial-by-trial save-spend choices and current sequence value. We also found planning activity, sequence valuations and tracking of sequence progress in medial and lateral prefrontal cortex and anterior cingulate cortex. Our results identify neural processes related to the formation and pursuit of internal economic saving plans and point to an important role for the human amygdala in planning for distant rewards. References: Grabenhorst, F., Hernadi, I., and Schultz, W. (2012). Prediction of economic choice by primate amygdala neurons. Proc Natl Acad Sci U S A 109, 18950-18955. Hernadi, I., Grabenhorst, F., and Schultz, W. (2015). Planning activity for internally generated reward goals in monkey amygdala neurons. Nat Neurosci 18, 461-469.

Disclosures: L. Zangemeister: None. **F. Grabenhorst:** None. **W. Schultz:** None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.06/W21

Topic: F.01. Human Cognition and Behavior

Title: Ventrolateral prefrontal activity reflects increases in object value induced by larger choice sets

Authors: *J. FUJIWARA[1], N. USUI[2], S. EIFUKU[1], T. IIJIMA[3], M. TAIRA[2], K.-I. TSUTSUI[3], P. N. TOBLER[4];
[1]Fukushima Med. Univ., Fukushima, Japan; [2]Tokyo Med. and Dent. Univ., Tokyo, Japan; [3]Tohoku Univ., Sendai, Japan; [4]Univ. of Zurich, Zurich, Switzerland

Abstract: The act of choosing between objects generally increases the subjective value of the chosen object. However, it is unclear whether this increase in value is due to having chosen the object or to simply owning the object after choosing it. In this study, we dissociated these two

possibilities by varying the size of the choice set under the assumption that this manipulation would increase choice-related value but have no impact on ownership-related value. In the scanner, participants selected options from choice sets of various sizes (1, 2, 4, or 8 options) and of differing value (low or high, based on pre-choice attractiveness ratings), whereby size and value were manipulated independently. After an option was selected, participants re-rated the attractiveness of the chosen option. By comparing post- with pre-choice ratings, we found that the subjective value of options chosen from larger choice sets increased more than that from smaller sets, and activation of the ventrolateral prefrontal cortex reflected this change in value. Control analyses showed that this revaluation effect is specific to the influence of set size and independent of the influence of ownership. These data further elucidate why we like things we have chosen ourselves.

Disclosures: **J. Fujiwara:** None. **N. Usui:** None. **S. Eifuku:** None. **T. Iijima:** None. **M. Taira:** None. **K. Tsutsui:** None. **P.N. Tobler:** None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.07/W22

Topic: F.01. Human Cognition and Behavior

Support: 5R01MH098023

Title: Neural Representation of Value of Information

Authors: ***K. KOBAYASHI**, M. HSU;
Univ. of California, Berkeley, Berkeley, CA

Abstract: In many types of decisions, an agent acquires some piece of information over the course of time, e.g. reading abstracts to select which posters to visit. Information is often associated with some cost (e.g. reading time), and the agent has to decide whether to acquire information by comparing its value against its cost. Because information in these cases has no intrinsic value, its value only comes from the ability to make a decision more adaptively in the future. According to decision theory, normative value of information is expected marginal gain from making an informed decision, and requires simulation of the self's choice and its expected utility under all possible contents of information. Although proper valuation of such pure information is fundamental to a variety of decision problems, we still know little about its neural basis. We conducted a behavioral experiment to examine the extent to which value of

information conforms to normative predictions, and an fMRI experiment to investigate neural representation of value of information. The experiments involved a series of gambles. Each gamble was consisted of two possible monetary outcomes, one gain and one loss, and their probability was (50%, 50%). Subjects chose whether to play each gamble. Next, we presented an additional piece of information for each gamble. When purchased, the information provided more precise prediction of outcomes by updating probability distribution, based on which subjects could choose whether to play the gamble. For example, the information could reveal that probability over outcomes was either (33%, 67%) or (67%, 33%), instead of (50%, 50%). We displayed potential probability distributions by the orientation of a partition line overlaid on a roulette wheel, and asked subjects to choose whether to buy the information at some monetary cost. Behaviorally elicited value of information was largely consistent with normative predictions; value of information was larger when more precise prediction were supplied, and when potential outcomes were larger. Critically, when the information would not change probability, most subjects reported it as valueless. From fMRI, we found that activation in medial prefrontal cortex and striatum was correlated with value of information. We also found a number of regions which activation was correlated with expected value of gambles. Critically, these two types of representation were overlapped in medial prefrontal cortex. These results suggest that value of information, which depends on simulation of own choice, shares some neurocognitive processes with other types of valuation, consistent with the "common currency" hypothesis.

Disclosures: K. Kobayashi: None. **M. Hsu:** None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.08/W23

Topic: F.01. Human Cognition and Behavior

Support: NIH NINDS NS054775

Title: Neuroanatomical correlates of economic rationality in aging - Testing GARP

Authors: *H.-K. CHUNG[1], A. TYMULA[3], P. GLIMCHER[2];
[1]Psychology, [2]Inst. for the Interdisciplinary Study of Decision Making, New York Univ., New York, NY; [3]Economic, Univ. of Sydney, Sydney, Australia

Abstract: The population of people above 65 years old is growing and work investigating their physical ability, cognitive function and decision making is of increasing importance. We previously (Tymula, et al., 2013) found that highly functional older adults are surprisingly likely to make fundamental choice errors in economic situations (more likely to violate "first-order stochastic dominance") compared with all younger subjects. The number of these errors increases even further as elders age. While such choices are clearly detrimental to older people's financial wellbeing, there is no biomarker for this "irrational performance" at the individual level, and insufficient evidence on the effects of neuroanatomical aging on decision making. In this study, we recruited healthy elders so as to relate the degree of error in their choice behavior to brain structure. We adopted the behavioral paradigm designed by Harbaugh and colleagues (2001) to estimate the number of violations of the generalized axiom of revealed preference (GARP) in each individual as a measure of these errors. In a typical choice from our design subjects chose one of two collections (bundles) of goods. For instance "2 boxes of milk and 3 pieces of chocolate" or "4 boxes of milk and 2 pieces of chocolate". Assessing many such choices, an economically rational agent must both obey transitivity prefer increasing amounts of any good to decreasing amounts of that same good (within reason). In sum, the task allows us to calculate the number and strength of violations of GARP to quantify the degree of irrationality in choice for each individual. We then relate this behavioral measure of economic irrationality to individual brain structure. Our goal is to use whole brain voxel based morphometry (VBM) analysis to determine where the gray matter volume correlates with the behavioral measure of irrationality in choice. In a recently published study we found that grey matter density in the posterior parietal cortex predicts risk attitudes with surprising accuracy. Our ex ante hypothesis is that thinning beneath some threshold in this area gives rise to economic irrationality. Our preliminary results indicate that the paradigm works well. Older subjects with what would be considered normal cognitive ability according to popular cognitive function scales, violate GARP at different rates allowing us to rank them based on the degree of economic irrationality in their choice. Ongoing imaging of 40 subjects should allow us to rapidly test our ex ante hypothesis.

Disclosures: H. Chung: None. **A. Tymula:** None. **P. Glimcher:** None.

Poster

081. Value-Based Human Decision Making

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Program#/Poster#: 81.09/W24

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH086492

Title: The diversity of distributed decisions

Authors: *M. E. WHEELER[1], E. J. PETERSON[2];
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Abstract: The integration of evidence over time is associated with accumulator signals in neural populations. Empirical and theoretical arguments suggest these accumulators act as general cortical computational mechanism, and therefore are a crucial component in decision making. Past work studying cortical responses in animals has focused on select frontal and parietal regions, showing that some neurons in these regions indeed behave similar to a formal drift diffusion model. However fMRI studies of evidence integration, which provide whole-brain measures of blood-oxygen-level-dependent (BOLD) signal, indicate that accumulation signals are widespread. These same fMRI studies also show that there are a variety of BOLD response profiles that appear to predict reaction time but do not take on the characteristic accumulator shape, defined here as a reaction-time-dependent ramping of activation as sensory evidence arrives and ceasing just prior to the commitment to a choice. The diversity of apparent reaction time predictive signals motivated this MVPA reanalysis of 5 fMRI studies spanning more than 80 participants. We report here our efforts to use the BOLD signal to predict reaction time. The two key results are, (1) while reaction time predictive signals are widespread in human cortex, the regions that are most predictive are highly task dependent and, (2) while accumulator-shaped time courses are important predictors of human behavior, many of the strongest signals took on a range of other profiles. Although direct neuronal recordings are needed to fully confirm our results, we conclude that the neural correlates of human evidential decision making are highly diverse, distributed, and task dependent.

Disclosures: M.E. Wheeler: None. **E.J. Peterson:** None.

Poster

081. Value-Based Human Decision Making

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Topic: F.01. Human Cognition and Behavior

Support: John Templeton Foundation Grant #36751

C.V. Starr Foundation Postdoctoral Fellowship

Title: Dorsal anterior cingulate and ventromedial prefrontal cortex have inverse roles in both foraging and economic choice

Authors: *A. SHENHAV, M. A. STRACCIA, J. D. COHEN, M. M. BOTVINICK; Princeton Neurosci. Institute, Princeton Univ., Princeton, NJ

Abstract: Research has grown increasingly interested in contrasting sequential foraging choices and economic choices between simultaneously presented options. Kolling et al. (2012, Science) [KBMR] proposed that these choice types are subserved by different circuits, with dorsal anterior cingulate (dACC) vs. ventromedial prefrontal cortex (vmPFC) driving foraging vs. economic choice. To support this account, they scanned human subjects while making a foraging choice between exploiting a current offer or swapping for potentially better rewards (Stage 1 of a trial [S1]) or an economic choice between two reward-probability pairs (S2). KBMR found different roles for dACC and vmPFC during the two task stages: in S1 dACC primarily tracked the relative value of foraging (RVF), and vmPFC the value of exploiting (especially when choosing to exploit); in S2 dACC tracked the relative similarity of chosen and unchosen values and vmPFC tracked their relative difference. We recently showed that dACC's role in S1 choices is better described by the difficulty of choosing (i.e., value similarity) than by RVF when correcting for choice biases and testing a sufficiently broad set of RVFs (Shenhav et al., 2014, Nat Neuro). Here we attempt to rule out a third possibility, that dACC tracks both choice difficulty and RVF. In doing so we address concerns about our study design that may have reduced sensitivity to an RVF effect (over and above a difficulty effect), in particular our use of (a) primarily numeric stimuli, (b) a free response paradigm (rather than an enforced delay to response), and (c) a moderate sample size (N=14). We further test an additional hypothesis regarding vmPFC: that its previously reported role in foraging may also be identical to its role in economic choice, and inverse to that of dACC. Specifically, we hypothesize that this region of vmPFC tracked the relative ease of choosing between the options presented. We scanned 31 subjects while they performed KBMR's task, including a response delay but using a wide range of RVFs and playing cards as reward-related stimuli. Replicating our previous findings, we find that dACC activity during both task stages is similarly well described by choice difficulty, and find no evidence of an RVF effect over and above this. Conversely, we find an inverse pattern in vmPFC, whereby activity during both task stages is associated with the ease of the current choice. The latter finding may reflect a value signal associated with choice ease, relative chosen value, or simply greater time off task prior to the response cue. Nevertheless, these findings further argue against the theory that foraging and economic choice are subserved by separate neural circuits.

Disclosures: A. Shenhav: None. **M.A. Straccia:** None. **J.D. Cohen:** None. **M.M. Botvinick:** None.

Poster

081. Value-Based Human Decision Making

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Program#/Poster#: 81.11/W26

Topic: F.01. Human Cognition and Behavior

Support: McDonnell Foundation Grant 5ro1ns06046

Title: Neural mechanisms of demand avoidance

Authors: *C. Z. SAYALI, B. CIULLO, D. BADRE;
CLPS, Brown Univ., Providence, RI

Abstract: When people are presented with two options of cognitive labor that differ in the level of effort they require, they choose the less demanding task. It has been observed that increased cognitive effort is associated with decreased responses in reward circuits but increased activity in areas related to cognitive control. Thus, it has been suggested that demand avoidance is a result of disutility associated with cognitive effort, and what makes a task more effortful is the presence of response conflict. However, these studies adopted categorical designs that rely on a subtractive approach which limits sensitivity to detect the shape of the behavioral and neural response functions. Therefore, the current study parametrically modulated the level of difficulty within a demand selection paradigm. Further, we separated choice from execution phases which allowed us to separate parametric responses driving decisions about what course of cognitive effort to take versus those related to execution of an effortful task itself. We hypothesize that what causes demand avoidance is the conflict associated with choosing the more effortful task. Therefore, we predict to see parametric changes in areas of medial prefrontal cortex related to conflict monitoring during the execution phase. Further, these responses should be related to decreases in ventral prefrontal and striatal reward systems during the choice phase. During the first phase of the experiment, participants performed multiple runs of 13 trials that varied according to six levels of difficulty which were modulated by the frequency of task-switching. Prior to each run of a given difficulty level, participants saw a symbolic cue that they selected to begin the run. In this way, each difficulty level came to be associated with a unique cue. In the second phase, participants were forced to choose which of two runs they wanted to perform by selecting between two associated symbols. They then complete the associated run of trials with the selected difficulty. The behavioral results have shown that participants consistently choose the easier task. The proportion of selecting the easier task increases linearly with increasing levels of difficulty difference between tasks. Also, selecting the more difficult task yields slower response times, indicating that choosing the more difficult task typically reflects a harder decision. Preliminary fMRI results show that MPFC tracks changes in selected difficulty. During

the execution phase, difficulty is tracked by a fronto-parietal network related to cognitive control.

Disclosures: **C.Z. Sayali:** None. **B. Ciullo:** None. **D. Badre:** None.

Poster

081. Value-Based Human Decision Making

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Program#/Poster#: 81.12/W27

Topic: F.01. Human Cognition and Behavior

Support: Department of Psychology, VSU

VSU College of Education and Human Services

Title: Risky decision-making in college smokers

Authors: *J. S. RODEFER;
Psychology, Valdosta State Univ., Valdosta, GA

Abstract: Cigarette smoking and tobacco-related illnesses result in approximately five million deaths around the world each year, with smoking being responsible for more almost one-half million deaths (approximately 20% of total) in the United States each year. Previously published research has suggested that smokers display poorer self-control as compared to non-smokers, and self-control training experience has been associated with greater success rates in smoking cessation. Yet, some data suggest that robust and reliable differences in risk-taking behavior do not exist between groups of smokers and non-smokers. The Iowa Gambling Task (IGT) is a computerized decision-making task that employs individual decks of cards that have different rewards and costs associated with each individual deck (A, B, C, D), and has been used with both clinical and non-clinical populations. One possible explanation for these data is that the association between smoking and risky decision-making may be related to the ability to delay gratification and resist temptation. Thus, a main goal of these experiments was to evaluate the hypothesis that magnitude of smoking deprivation contributed to risky decision-making. The present experiments examined both pattern of risky decision-making performance and specific deck selection in undergraduate smokers and non-smokers that were recruited from a university setting. Smokers were asked to either abstain or not abstain from smoking overnight before coming into the laboratory the following day. Adherence to abstinence request was ascertained by measuring CO levels in exhaled breaths. The risky decision-making performance was divided

into early learning (trials 1-40; learning) and later performance (trials 41-100; performance) blocks of trials for each of the individual decks. Individuals who successfully abstained from smoking overnight chose more frequently from riskier decks during the later blocks of trials. There was a general trend for increased choice from suboptimal desks in later trials. In contrast, smokers who did not abstain demonstrated an increased preference for decks representing optimal decision-making. When taken in sum, these data suggest that nicotine abstinence, as measured by CO levels, may contribute to risky decision making in the IGT.

Disclosures: J.S. Rodefer: None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.13/W28

Topic: F.01. Human Cognition and Behavior

Support: NIH R01AG039283

Title: Episodic memories predict adaptive value-based decision-making

Authors: *L. E. HUNTER[1], O. FELDMANHALL[1], V. MURTY[1], L. DAVACHI[1,2], E. A. PHELPS[1,2,3];
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Abstract: Prior research illustrates that memory can guide value-based decision-making. For example, during reinforcement learning, the value associated with an option is incrementally learned across multiple exposures, suggesting that memories guide stimulus response learning. However, other types of memories, such as episodic recollections, may also contribute to value-based decision-making. Using a novel behavioral paradigm, here we test to what extent value based decision-making is influenced by episodic recollections for past rewarding experiences (i.e. stimulus-reward associations). Participants completed a task where they first learned the value associated with lotteries. After a short delay, they completed a decision-making task in which they choose whether they wanted to re-engage with previously encountered lotteries. Finally, participants completed a surprise memory test for the lotteries and their associated values. Results indicate that participants chose to re-engage more often with lotteries that resulted in high versus low rewards. This type of adaptive decision-making is only seen when individuals have contextually embedded memories for the reward values associated with

individual lotteries, suggesting that adaptive decision-making is associated with detailed, episodic recollections of prior experiences. Further, we find that this reliance on episodic memory for adaptive decision-making generalizes to more complex, ecologically valid choice behavior, such as social decision-making. These findings provide an important integration of declarative memory and decision-making literatures to better understand key mechanisms supporting adaptive behavior.

Disclosures: L.E. Hunter: None. **O. FeldmanHall:** None. **V. Murty:** None. **L. Davachi:** None. **E.A. Phelps:** None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: John Templeton Foundation: University of Chicago Wisdom Research Grant

Title: Market experience attenuates the endowment effect through modulation of anterior insula: A training study

Authors: *L. TONG[1], K. J. YE[2], H. C. NUSBAUM[1], A. HORTACSU[2];
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Abstract: Consumers ask a greater price for goods that they own than they are willing to pay for otherwise identical goods, a phenomenon known as the endowment effect. Previous fMRI research indicates that lower insula activity during selling is related to a reduced endowment effect in experienced compared to inexperienced traders. The present study extends this research by experimentally manipulating market experience. Inexperienced traders (n=13) made trading decisions while undergoing fMRI before and after the treatment of short-term selling experience. In a pretest session prior to market experience, and in a posttest session after the market experience treatment, participants made a series of decisions to buy or sell products at high and low prices during neuroimaging. Between the two scanner sessions, participants were given incentives to trade consumer goods on eBay over the course of two months. Trading experience reduced both the endowment effect behaviorally ($p<0.05$), and right anterior insula activation when presented with lowball offers during selling ($p<0.05$). Moreover, insula activity during low selling offers positively predicted the endowment effect, and partially mediated the effect of trading experience on the endowment effect. These results provide causal evidence that selling

experience reduces the salience of selling at a loss, consistent with behavioral evidence that market experience attenuates economic biases.

Disclosures: **L. Tong:** None. **K.J. Ye:** None. **H.C. Nusbaum:** None. **A. Hortacsu:** None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.15/W30

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant K12HD073945

Title: The effects of incentive valence on instrumental performance

Authors: ***J. GALARO**, P. CELNIK, V. S. CHIB;
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Abstract: Incentive mechanisms reward favorable performance and punish unsuccessful performance. In this experiment we examined how prospective monetary gains and losses influence both motor cortical excitability and instrumental performance. During the experiment participants were presented with possible gains or losses of different magnitudes, where payout was contingent on successful performance of a motor reaction task involving a simple, visually cued movement of the finger. Behavioral heuristics (e.g. reaction time) were computed to measure performance vigor. Our behavioral results indicated that participants exhibited increased vigor for increased reward magnitude in both the gain and the loss domain. Moreover, we found a more pronounced increase in vigor metrics for losses as compared to gains, which was related to participants' differential subjective valuation of gains compared to losses (i.e. individuals' loss aversion). Further analyses will examine the relationship between motor cortical excitability and reaction time and subjective preferences for incentive. Specifically we will test if motor cortical excitability mediates relationships between individuals' subjective preferences for incentives and resulting motor performance and vigor.

Disclosures: **J. Galaro:** None. **P. Celnik:** None. **V.S. Chib:** None.

Poster

081. Value-Based Human Decision Making

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Program#/Poster#: 81.16/W31

Topic: F.01. Human Cognition and Behavior

Support: Swiss National Foundation #31003A_143707

Title: Why do we take so long when faced with two good options?

Authors: *J. DRUGOWITSCH, S. TAJIMA, A. POUGET;
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Abstract: In every-day ambiguous and noisy environments, decision-making requires the accumulation of evidence over time. In perceptual decision-making tasks (e.g., discriminating a motion direction), choices and reaction times are well-fitted by drift diffusion models (DDMs). These models represent the accumulated belief about the difference in sensory evidence as the location of a diffusing particle that triggers a decision once it reaches one of two decision boundaries. Recently, DDMs have been shown to also fit human behavior in value-based decisions, where subject compares the endogenous values of possible options (e.g., deciding between two lunch options). In this case, the DDMs only represent the difference in values, such that they predict behavior that is insensitive to the absolute values of the compared options. This is counterintuitive because, intuitively, choosing between two highly-rewarded options will yield high rewards for either choice, such that these choices should be made more quickly to save time and effort. To resolve this conundrum, we sought for the theoretically optimal strategy to maximize the reward rate per unit time in such value-based decision paradigms. Using dynamic programing, we found that this optimal strategy indeed reduces for simple, but realistic, scenarios to a particular class of DDMs that feature “collapsing boundaries”. That is, the distance between the decision boundaries shrinks over time, thus urging a decision. Interestingly, the speed of boundary collapsing is greatly modulated by the a-priori reward distribution. If the two options are highly rewarded on average, the boundary should collapse more rapidly. This collapse ensure that average reaction times depend on the absolute values even if decisions within individual trial are only guided by the relative value between options. Moreover, the speed of the boundary collapse for value-based decision tends to be larger than the ones that is optimal for standard perceptual decision tasks in which subjects try to maximize the number of correct answers. These results clarify the difference between value-based and perceptual decisions, and may reconcile the ongoing debate on when subject should have collapsing boundary in decision-making.

Disclosures: **J. Drugowitsch:** None. **S. Tajima:** None. **A. Pouget:** None.

Poster

081. Value-Based Human Decision Making

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Program#/Poster#: 81.17/W32

Topic: F.01. Human Cognition and Behavior

Support: NIH grant MH101592

Title: Value-based biasing of decision variable dynamics under speed pressure

Authors: K. AFACAN[1], A. BLANGERO[2], *S. KELLY[3];
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Abstract: Sensory-instructed behaviors are biased in favor of actions that are more valuable than the alternatives. Under situations of intense speed pressure where the time available to act is short with respect to the timeframe over which sensory selectivity grows to a level sufficient for accurate perception, such biases become central to adaptive strategies for reward maximization. Cognitive modeling work based on the principle of sequential sampling provides a natural account of how such prioritization takes place: a "decision variable," which diffuses between two alternative decision bounds with a drift tendency governed by sensory evidence, is started closer to the more valuable bound. In this project we provide behavioral and neural evidence for a distinct mechanism of prioritization, whereby the decision variable is not just started closer to, but initially, actively launched toward, the more valuable decision bound, and is then dynamically re-routed by the incoming, discriminatory sensory information. In our task, subjects manually reported on a two-alternative fixation color change using their left and right hands within a very strict deadline, with more points rewarded for correct discrimination of one color than the other. A model in which drift rate was subject to the additive influence of linearly-growing sensory evidence and a constant value-based bias provided a closer fit to reaction time data than more typical starting-point bias or constant drift rate models. Further, lateralized motor preparation signals in the human event-related potential exhibited a characteristic "turn-around" in trajectory for slow, correct, low-value actions as predicted by the model. Ongoing analyses are being conducted to explore inter-individual differences and potential mixed strategies that combine starting point and drift biases.

Disclosures: K. Afacan: None. **A. Blangero:** None. **S. Kelly:** None.

Poster

081. Value-Based Human Decision Making

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Topic: F.01. Human Cognition and Behavior

Support: NSF ENG-1137279 (EFRI M3C)

Title: Investigating cortical networks to characterize neural dynamics of reward based decision making

Authors: *H. COURELLIS[1,2], J. IVERSEN[2], D. PETERSON[3], H. POIZNER[3], G. CAUWENBERGHS[1,3];
[1]Bioengineering, [2]Swartz Ctr. for Computat. Neurosci., [3]Inst. for Neural Computation, UCSD, La Jolla, CA

Abstract: Reward based decision making is a complex process that requires the participation of both cortical and subcortical networks and involves dopaminergic neurons in the basal ganglia connecting to structures in the frontal lobe to drive higher level decision making processes. To investigate cortical networks associated with reward based decision making, we analyzed the EEG of individuals performing a reward based decision task, identified Regions Of Interest [ROIs] (and their corresponding anatomical structures), and examined causal relations among them. 64 channel EEG was collected by Peterson et al. (Neuroscience 163 (2009) 1092-1101) from 11 healthy individuals of ages of 58-72, while they performed a task that tested their ability to make reward based decisions. Between two images on a screen, they selected the image that would give them more money using their knowledge from previous decisions. First the reward based decision making cortical network of a single participant was investigated. The individual's EEG data were analyzed employing Independent Component Analysis and subsequent identification of causal interactions among the resulting Independent Components (ICs). A functional decision making network was identified, linking the visual, anterior cingulate, and motor cortices in a manner consistent with the requirements of the image selection task performed by the participant: visualizing the images, making a decision, and reaching to select the chosen image. Subsequently, EEG data from all 11 participants were analyzed and generalized ROIs were identified, populated by clusters of ICs drawn from the group's EEG data. IC clustering features, which included scalp maps, Event Related Potentials (ERPs), Event Related Spectral Perturbation, Inter-Trial Coherence, power spectra, and dipole locations were preprocessed using principle component analysis. A competitive layer neural network was employed to perform the clustering that resulted in 12 IC clusters. Average ERPs for each resultant cluster and cluster tightness were used to identify ROIs and their correspondence to

anatomical structures potentially relevant to the decision making network was identified. The main structures of interest were the Anterior Cingulate, Orbitofrontal, Prefrontal cortices, and the Inferior Frontal Gyrus, all of which have been associated with the activity of decision making networks. Activity in these ROI's is determined through anatomically constrained low resolution electromagnetic tomography, generating source activations in the same locations for all participants, thus allowing for causal connections between these ROI's to be evaluated.

Disclosures: H. Courellis: None. **J. Iversen:** None. **D. Peterson:** None. **H. Poizner:** None. **G. Cauwenberghs:** None.

Poster

081. Value-Based Human Decision Making

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Program#/Poster#: 81.19/W34

Topic: F.01. Human Cognition and Behavior

Support: 1R01MH104251-01A1

Title: Value aftereffects: evidence for dynamic adaptation in the valuation process of human subjects

Authors: *M. KHAW, P. W. GLIMCHER, K. LOUIE;
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Abstract: While standard theoretical frameworks for decision-making assume stable and unchanging preferences, empirical observations of context-dependent choice behavior suggests a relative process of valuation. Recent evidence points towards a flexible and adaptive value coding in neural systems, scaling to the range of recent rewards (Padoa-Schioppa, 2008) or the value of alternative offers (Louie et al., 2011). Context effects driven by aspects of the current choice set are well-characterized by value normalization (Louie et al, 2013); however, whether analogous temporal value context effects exist in human behavior is unknown. Here, we investigate bidding behavior with a value-based adaptation manipulation. In sensory processing, classic adaptation paradigms elicit history-dependent changes in perception. For instance, prolonged viewing of curved lines biases subsequent judgments of concavity, and prolonged viewing of visual motion produces an opposite motion aftereffect in subsequent static stimuli (the waterfall illusion). Analogous to visual aftereffects, we hypothesize that subjective valuations *shift* after prolonged exposure of the subject to different value quantities. The experimental protocol consisted of two adaptation blocks interspersed between three test auction

blocks. 44 adults (24 female, mean age = 23.43 years) participated. During bidding blocks, subjects were asked to declare a willingness-to-pay (WTP) for snack items via an incentive-compatible Becker, Degroot & Marschack auction. During adaptation blocks, subjects were asked to evaluate repeatedly (300 trials per block) the pleasantness of either high- or low-value snack items (as determined from the first bidding block). Consistent with a temporal form of value normalization, we find that bids following a history of high-value adaption are lower on average than bids following low-value adaptation. The bids themselves exhibit a regression toward the mean from the very first block as trials elapse. A linear regression of bid deviations on past bid values reveals a significant positive effect of the last immediate bid. In contrast, there is a significant negative effect of a longer-running aggregate of past values. These relationships also appear to vary over time, with the negative effect of a long-running average being strongest at the start of bidding blocks, and vice versa for the effect of immediately past bids. Our results indicate that the subjective valuation of goods - much like perceptual judgments - is dynamically affected by the recent history of experiences. Thus, adaptation may be a general phenomenon across both sensory and valuation domains.

Disclosures: **M. Khaw:** None. **P.W. Glimcher:** None. **K. Louie:** None.

Poster

081. Value-Based Human Decision Making

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Topic: F.01. Human Cognition and Behavior

Support: BMBF Grant 01GQ1006

Title: The representational space of value-based decisions during model-based and model-free reinforcement learning

Authors: *M. N. HEBART, G. E. WIMMER, J. GLÄSCHER;
Dept. of Systems Neurosci., Hamburg, Germany

Abstract: A major line of research in decision neuroscience is aimed at characterizing the computational and neural principles of the learning systems that underlie human and animal choice behavior. Previous seminal studies have focused on identifying model-derived neural computations of model-based learning (Gläscher et al., 2010, Daw et al, 2011) and have demonstrated the existence of an efficient neuronal algorithm for learning state transitions in complex environments. What remains unknown is the exact neural representation of these

complex state spaces that are shaped by such computations. Here we suggest that probabilistic state spaces can be characterized in terms of representational distance, which enables humans to choose the action that is representationally the closest to the desired state. To investigate this principle, we devised a modified version of the popular two-step task (Daw et al., 2011), varying transition probabilities between initial and terminal states and reward values of terminal states. Bayesian model comparison revealed that participants' behavior could be best explained by hybrid model-based and model-free learning with eligibility traces and a persistence parameter biasing softmax action selection. Using multivariate pattern decoding, we identified representations of states in visual cortex as well as other cortical brain areas. Building up on these findings, we used pattern-component modeling (Diedrichsen et al., 2011) - an extension of representational similarity analysis - to deconstruct the representational similarity between initial and terminal states into components of (1) state transition probability, (2) expected reward value, and (3) their interaction. Our results demonstrate separable representations of each of these components in the human brain, suggesting that humans may base their model-based choices on an integrated representation of state-state and action-state similarity. Our approach provides a first step in identifying the neural foundation of complex state representation and the forces that shape them.

Disclosures: **M.N. Hebart:** None. **G.E. Wimmer:** None. **J. Gläscher:** None.

Poster

081. Value-Based Human Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 81.21/W36

Topic: F.01. Human Cognition and Behavior

Title: Neural mechanisms supporting persistent maladaptive food choices

Authors: ***K. E. FOERDE**[1], J. STEINGLASS[2,3], D. SHOHAMY[4], B. T. WALSH[2,3];
[1]Psychology, New York Univ., New York, NY; [2]Div. of Clin. Therapeut., New York State Psychiatric Inst., New York, NY; [3]Psychiatry, Columbia Univ. Med. Ctr., New York, NY; [4]Psychology and Kavli Inst. for Brain Sci., Columbia Univ., New York, NY

Abstract: People routinely make poor choices, to the detriment of their health and well-being. Yet, despite knowledge of the negative consequences, maladaptive choices persist. Such behavior is particularly common in choices about which foods to eat, but the neural mechanisms that support persistently poor food choices and how they relate to everyday behavior are unknown. We examined these issues in individuals with Anorexia Nervosa (AN), whose

persistent choice of low calorie foods poses a serious and sometimes fatal threat. Participants with AN were receiving treatment aimed at weight restoration to a medically healthy weight, and were tested within 24 of hours of hospitalization. We used functional magnetic resonance imaging (fMRI) to examine brain activity while individuals with AN and healthy controls made decisions about which foods to eat. The task comprised three phases. In the first two phases, participants rated the healthiness and the tastiness of high- and low-fat food items. Next, in the third phase, each participant was asked to make a series of choices between a reference item, randomly selected among items rated by the individual as neutral in both the health and taste rating phases, and each of the other foods. Participants were informed that, immediately following the task, they would be served one of their choices as a snack and were expected to consume it - creating a real-world contingency for their choices. This paradigm allowed an individual's ratings of food to determine the reference item, which was critical in comparing behavior of healthy controls with individuals with AN, as the two groups were expected to rate the health and tastiness values of food differently. To determine whether choice responses in the task were related to actual eating behavior, we compared responses on the food choice task with caloric intake in a lunch-time meal the next day in which participants were presented with a buffet-style array of foods and asked to eat whatever they wished. Food was weighed before and after and amount consumed in grams and kcal was calculated. Using fMRI, we found that the dorsal striatum was more strongly associated with food choices in AN relative to controls, whereas ventral striatal activity did not differ between groups. Moreover, fronto-striatal connectivity during the experiment predicted the amount of food consumed in a meal the next day. These findings demonstrate that activity in fronto-striatal brain circuits is related to the persistent restrictive intake seen in AN, and may relate to persistent maladaptive behavior more broadly.

Disclosures: **K.E. Foerde:** None. **J. Steinglass:** None. **D. Shohamy:** None. **B.T. Walsh:** None.

Poster

082. Social Decision Making

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Support: 1R01DA038063-01

Title: A systematic comparison of models of generosity

Authors: *C. DI TELLA, P. GLIMCHER, W. MA;
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Abstract: Objective: Decisions that affect other people's outcomes are a fundamental aspect of social behavior. In non-strategic contexts, such decision-making processes are formalized as models of "other-regarding preferences", which measure how much a person is willing to sacrifice to help or hurt others. Many models of other-regarding preferences have been proposed, but there has not been convincing evidence in favor of any particular one. Moreover, MRI studies of other-regarding preferences typically assume a model without justifying their choice. The goal of our project was to empirically identify the best model from amongst the leading models of other-regarding preferences, using optimal trial design and Bayesian model selection procedures. In addition, we assessed the effect of social distance on the parameters of the other-regarding preferences. Methods: We conducted two studies consisting of an incentive-compatible modified dictator game with two possible recipients, one a friend and one a stranger. Previous studies of generosity have handpicked trials believed to be informative about models or parameters. Here, we took a more systematic approach. In Study 1, we selected trials to maximize mutual information between the data and the model, i.e., our ability to determine which of three classes of models (Charness-Rabin, Cobb-Douglas and Rawlsian) best accounted for the behavior of our subjects. In Study 2, we selected trials to maximize mutual information between the data and the parameters within the best fitting model in our population. Results: Study 1: Several model comparison measures identified the Charness-Rabin model as by far the best descriptor for our population. The Charness-Rabin model posits a linear utility function, with a weight on the other's welfare that depends on whether the decision maker is better or worse off; the Fehr-Schmidt model is a subclass of Charness-Rabin. Study 2: This study revealed that, regardless of context (better or worse off), the weights placed on the welfare of a friend are higher than those placed on the welfare of a stranger. Moreover, the pattern of weights by context is markedly different for friends and strangers: subjects tend to display classical difference aversion with strangers, with negative weights when the subject is worse off, and positive weights only when better off. By contrast, the weights for friends tend to be always positive, albeit lower when worse off than when better off. We interpret these results as evidence of a tendency towards prosocial difference aversion when it comes to friends and antisocial difference aversion when it comes to strangers.

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Poster

082. Social Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 82.02/W38

Topic: F.01. Human Cognition and Behavior

Title: Gender difference in the performance of cheater-detection in social exchange: an eye-tracker study

Authors: *M. WATABE[1], Y. UEDA[2], T. KATO[3], M. SHINADA[4], T. YAMAGISHI[5]; [1]Monash Univ., Bandar Sunway, Malaysia; [2]Kyoto Univ., Kyoto, Japan; [3]Kyushu Univ., Fukuoka, Japan; [4]Tokyo Gakugei Univ., Tokyo, Japan; [5]Hitotsubashi Univ., Tokyo, Japan

Abstract: Introduction: Cheater detection (CD) is a cognitive ability to find free-riders in social exchange. CD has been considered as one of the most essential abilities for humans to survive as social groups (Cosmides Cognition, 1989). Experimental studies have demonstrated the existence of this ability across cultures and ethnicities, but individual differences of this ability has been unexamined. Our previous research (Watabe et al., 2014) has suggested that the detection ability would differ between males and females. It revealed that male participants show higher detectability for male cheaters than female participants. In this study, we replicated this experiment with an eye-tracker system to investigate more detailed process of cheater detection performance. **Method:** We herein collected 18 male and 17 female university students' data and investigated how they detect cheaters by watching self-introduction video with cheaters and cooperators. Participants watched video streaming, a pair of same-sex persons (one is a cheater and the other is a cooperator) appeared simultaneously on the display, introducing themselves without audio for 30 seconds (Shinada et al. 2010). Then, participants were asked to detect which one is the cheater. They did this task for 36 pairs (18 male pairs and 18 female pairs). **Results:** The results show again that male participants show higher detectability for male cheaters than female participants. According to the analysis of the eye-tracking data, males who spent longer time to look at the cheater's face showed higher detectability, and female who spent longer time to look at the eyes of videotaped persons showed lower detectability. We also found that females who spent longer time to look at the mouth (and around) showed higher detectability. **Discussion:** The present results confirmed the previous findings of gender difference on CD performance. Also, the results of eye-tracking data suggest that males and females adopt different strategies for detecting cheaters. Males tend to look at the target's face overall whereas females tend to look at parts of the target's face. The female's detectability would depend on which part of the face she focus on.

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Poster

082. Social Decision Making

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Topic: F.01. Human Cognition and Behavior

Support: Duke Institute for Brain Sciences Incubator Award

Title: Mechanisms of decision making in criminal trials

Authors: J. R. LAW[1], J. A. G. SKENE[1], J. M. PARELMAN[4], D. H. BESKIND[2], N. VIDMAR[2], R. M. CARTER[5], J. M. PEARSON[3], *J. SKENE[1];
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Abstract: During a criminal investigation and trial, decisions by police officers, prosecutors, judges, and jurors are governed by established procedures and rules. These procedures reflect well-accepted assumptions about how humans weigh evidence about the guilt or innocence of a defendant. However, research has shown that traditional models of decision-making cannot adequately account for the behaviors actually observed during complex cognitive tasks. Here, we demonstrate an experimental task that quantifies the effects on judgments of guilt of factors commonly seen in criminal prosecution. Using descriptions of crime scenarios that differ in evidentiary and potentially biasing content, we asked participants to rate the likelihood of guilt and the punishment deserved for each scenario. We targeted two populations, representing different participants in the legal system: potential jury members (assessed using MTurk participants) and legal professionals (assessed in a law student population). We then used a generalized linear mixed model to evaluate the effect of each type of evidence and each crime type on ratings of guilt and deserved punishment. Across populations, we found that the accumulation of evidence linking a defendant to a crime was the strongest determinant of confidence in guilt. DNA and other physical evidence had the largest effects, with a lower effect for eyewitness testimony. In contrast, prescribed punishment correlated strongly with the type of crime, but was minimally affected by the amount of evidence implicating the accused. Surprisingly, prior convictions for a related crime_conventionally viewed as strongly prejudicial for criminal defendants_had only a modest influence on judgments of guilt. While the results were similar for law students and MTurk participants, law students showed a significantly lower overall confidence in guilt and larger effects of physical evidence and witness testimony. Although the legal system seeks to determine guilt solely on the basis of evidence, we found that type of crime significantly influenced confidence in guilt, independent of evidence linking the defendant to the crime. This baseline confidence was positively correlated with subject ratings of the amount of punishment each crime deserved. Previous studies have shown that moral

judgments of deserved punishment may be mediated by a neural circuit that integrates perceived threat or harm to the victim with the intent of the perpetrator (theory of mind). Our results suggest that elements of this "punishment" circuitry can modulate processes involved in the rational evaluation of evidence directly implicating someone accused of a crime.

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Poster

082. Social Decision Making

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Support: VR 20121999

Title: The effect of testosterone on the Ultimatum Game: A single-dose administration study

Authors: *E. KOPSIDA, J. BERREBI, P. PETROVIC, M. INGVAR;
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Abstract: In recent years studies on the Ultimatum Game have successfully shown that human beings forfeit, under certain circumstances, the rule of profit maximisation and act contrary to their financial gain, by rejecting unfair offers. A possible explanation for this behaviour could be that unfair offers elicit negative emotions to the responders, who punish the unfair proposer by rejecting the offer. Neuroimaging studies have shown that unfair offers are associated with activity in insula, anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (dlPFC), and amygdala. A potential role of testosterone on the Ultimatum Game has also been proposed, albeit with inconsistent findings. In the present study, we sought to further investigate the role of amygdala and testosterone on the Ultimatum Game, by conducting a double-blinded, single-administration study (N=68; 28 women). 60mg of Tostrex was administered to male and female healthy volunteers, three hours prior to undergoing an fMRI session, during which they played a standard version of the Ultimatum Game. This dose was sufficient to significantly increase participants' testosterone levels compared to placebo ($Z=-5.755$, $p=.000$). The behavioural analysis revealed a statistical trend, as participants in the testosterone group tended to accept a greater number of unfair offers than participants in the placebo group ($Z=-1.483$, $p=.138$), irrespectively of gender. The main contrast unfair>fair offers yielded activation in areas previously associated with the Ultimatum Game ($p<.05$, FWE). To test our primary hypothesis

that testosterone would increase amygdala activation in relation to unfair offers, the contrast unfair>fair offers was compared between groups. A non-significant trend towards greater bilateral amygdala activation on the testosterone group was observed (left: Z=1.86, p=.03, uncorrected; right: Z=1.79, p=.03, uncorrected). Furthermore, the testosterone group displayed a greater activation in the right dlPFC (Z=4.07, p=.000, uncorrected) than the placebo group. Finally, a non-significant trend towards higher left amygdala activation was shown for rejection of unfair offers compared to acceptance (Z=2.10, p=.018, uncorrected). The present study was the first to investigate the effect of testosterone on both genders using the same experimental protocol. The findings indicate that testosterone administration could be related to a greater acceptance of unfair offers, potentially by acting on the dlPFC.

Disclosures: **E. Kopsida:** None. **J. Berrebi:** None. **P. Petrovic:** None. **M. Ingvar:** None.

Poster

082. Social Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 82.05/W41

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 26120732

Title: Making better decisions by predicting others' minds

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Abstract: In everyday life, humans often not only predict what others decide but also use the predictions for making their own better decisions. It is so essential in social cognition and how they predict others are often examined, however, how the prediction is used for better decisions remains elusive and also, what the underlying neural mechanisms and computations are. To address this issue, we conducted human fMRI, devising a novel experimental paradigm, combined with computational modeling. The paradigm is composed of three tasks, building on value-based decision-making (choosing one of two options for maximizing their monetary rewards). In main task, the subject did value-based decisions for oneself, but each option's value

changes based on others' choices, so that the task required one to decide based on the prediction of others' decisions. Two control tasks examined each component in the main task; subject performed ordinary value-based decisions (self task) and also predicted others' decisions (other task). This setting allowed us to construct models of the two components and then build a non-parametric model to examine how they were combined for the choices in the main task. Using different models for different hypotheses, we had three findings in behavior (n = 24). First, we found that the subject's choice behavior is certainly modulated by their predictions of others' decisions. Second, they used the prediction in a particular way, that is, generating hypothetical sets of the subject's options based on the prediction, i.e., somewhat in a Bayesian way. Third, however, their behavior is further modulated by the attraction or interaction over the options between the hypothetical sets, leading to their actually suboptimal decisions. These results were further confirmed by computational models with free parameters which account for individual difference in decision-making. We are currently collecting and analyzing human fMRI data, using model-based analysis. Our preliminary results (n = 13) showed differential brain circuits for predicting the others (in other task) and using the prediction for better decisions (in main task). These findings together indicate the hierarchical mechanisms from predicting the others to making better self-decisions, using the predictions.

Disclosures: **N. Ma:** None. **N. Harasawa:** None. **K. Ueno:** None. **N. Ichinohe:** None. **M. Haruno:** None. **K. Cheng:** None. **H. Nakahara:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 26120732

Title: Neural mechanisms for deciding with rewards to others

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Abstract: Human makes decisions guided by one's reward expectation, but also in social setting, adjusted frequently in favor for rewards to others. This nature leads to fundamental debates in the social sciences, such as altruism. A large literature exists on the debate, however, evidence is scarce for a perhaps simpler and basic question in neuroscience, that is, how the others' reward takes part in the process of one's own reward-based decision-making and what the neural mechanisms are, especially whether they are social-domain specific or non-specific. To address these issues, we combined human fMRI with computational modeling in reinforcement learning paradigm. Our experimental task is composed of three conditions (three types of trials.) In standard condition, the subjects performed ordinary value-based decisions between two options, each of which is associated with reward probability and magnitude. In other and bonus condition, extra reward to others and the self was attached to either option and it was always given when the option was chosen. In behavior, we found that others' reward influenced the choice behavior in the subjects' majority, although the influence is weaker than that by bonus in the same face amount. Using logistic regression in behavior, we quantified each value (standard, others', and bonus value) and decision value (final value difference between the options including extra values). Using the quantification, we analyzed BOLD signals for identifying neural correlates of each and decision values, and the processes to integrate others' value into decision value. First, the significant neural correlates of both others' and bonus values are found in the dorsomedial and dorsolateral prefrontal cortices (dm/dlPFC). But the correlate of only others' value is found in right temporoparietal junction (rTPJ). Decision value, and standard value difference, is found in the ventromedial prefrontal cortex (vmPFC). Second, by further analyses on others' value, we found that the right dlPFC and dmPFC activation is elevated particularly when the option with others' reward is chosen and not chosen, respectively, whereas the rTPJ activation does not distinguish the choices. Third, psychophysical interaction analysis indicated that the vmPFC activation is significantly correlated with those in rTPJ and right dlPFC when decision value involves others' reward. Taken together, these findings demonstrate neural pathways for encoding others' reward and using it for a final decision, in parallel to but also uniquely from that for processing bonus, or self-additional reward.

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Poster

082. Social Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 82.07/W43

Topic: F.01. Human Cognition and Behavior

Title: Neural correlates of creative thinking

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Abstract: Creative thinking is connected to various mental processes, including relaxation of mental constraints and activation of memory networks. In this study, we focus on analytical and verbal processes, especially the intuitive and nonverbal processes, involved in creative thinking, and clarify the underlying neural mechanisms. Eight healthy experimental participants underwent functional magnetic resonance imaging (fMRI) while they carried out creative thinking tasks requiring them to conceive creative ideas. Verbal- and image-recall tasks were also performed for comparison. A significant activation was observed in the left inferior frontal gyrus (BA44) during the verbal-recall compared with the image-recall task. In addition, a significant activation was observed in the superior occipital gyrus (BA19) during the image-recall compared with the verbal-recall task. On the other hand, a significant activation was observed in the left middle temporal gyrus, angular gyrus, precuneus, and lingual gyrus during creative thinking tasks compared with the verbal and image-recall tasks. Thus, our results clarify the relationship of verbal and nonverbal processes with creative thinking.

Disclosures: **K. Ueda:** None. **R. Hikichi:** None. **M. Nakao:** None. **T. Noda:** None.

Poster

082. Social Decision Making

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Program#/Poster#: 82.08/W44

Topic: F.01. Human Cognition and Behavior

Support: NSFC 31470995

Title: Same costs cost different: the effect of prosociality on third-party punishment using feedback-related negativity

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Abstract: While in dictator games a third party can give costly punishment to the dictator when confronted with unfair distribution, previous studies have found that inequality aversion and reciprocal altruism might account for this. However, little research has focused on people who are rarely inclined to give third-party punishment. Prosociality as an individual difference determines behavior in economic games and many real-life situations. This study investigates how prosociality (prosocial or individualist) influences third-party punishment. Event-related potential (ERP) was collected to explore the neural correlates of outcome evaluation on allocation offer. We used a behavioral task to identify people with different prosociality. Sixteen prosocials and sixteen individualists took part in the ERP experiment. In the adapted dictator game, Player A had an endowment of 100 points and would transfer 10 to Player B, who had no endowment. Participants, the third party, were endowed with 50 points, and had the option of punishing Player A with 5 or 15 points (the cost was decided by the computer) after the observation. Observing the distribution outcomes (90:10), participants could punish player A with two costs, which resulted in a fair distribution (50:50). For feedback-related negativity (FRN) analysis, we measured the average amplitude in the 250-350 ms time window for distribution feedback that was localized to frontalcentral electrodes (Fz, FCz, Cz). Behavioral results found that a lower cost caused more third-party punishments. Prosociality influenced third-party punishment: facing unfair offers, prosocials made more costly punishments than individualists. For prosocials, there was no obvious difference between their punishments at different cost levels. However, individualists' made more third-party punishments when costs were less. ERP results revealed that FRN was modulated by prosociality. For both prosocials and individualists, low costs elicited a significant larger FRN than high costs. On the same high cost condition, however, prosocials elicited larger FRN than individualists. Results were interpreted in terms of expectancy deviation theory: more negative amplitudes are presumed to be related to paying more attention to dictator's allocation and outcome. The current results suggest that high cost leads to less attention to dicator's allocation and outcome for individualists because they like to maximize the sum of resources for oneself, whereas both high and low cost do not decrease prosocials' attention to dictator's allocation and outcome because they prefer to maximize resources for oneself and others.

Disclosures: Y. Cheng: None. **C. Qu:** None.

Poster

082. Social Decision Making

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 82.09/W45

Topic: F.01. Human Cognition and Behavior

Title: Differences in people choosing cooperatively versus competitively during social dilemmas: an EEG spectral analysis

Authors: *A. J. WILSON[1], B. S. ROBINSON[2], N. J. A. WAN[2], K. E. JORDAN[2];
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Abstract: The neural intricacies of cooperation between humans is limited. The Prisoner's Dilemma (PD) has been used to study behavioral differences in cooperation and competition for decades, but only a small amount of research has been produced about the neural correlates of cooperation and competition within the framework of PD. Furthermore, other social dilemma games, such as Snowdrift (SD), have also been studied from a behavioral standpoint, but far fewer studies have researched the neural differences between people within SD. In this study, we investigate the differences in choice between PD and SD and also between people who are behaviorally cooperative and behaviorally competitive within these games. Since SD is a less risky social dilemma game in comparison to PD, we expect to find differences between games in choice behavior and level of neural activation recorded by EEG. Furthermore, we expect to find neural differences between people who are cooperative than people who are competitive. We analyze activation in alpha (8-12Hz) and beta (12-24Hz) within our regions of interests (ROIs): the prefrontal cortex (PFC) and inferior parietal lobule (IPL). We found behavioral differences between PD and SD, where people cooperate more during SD (~50%) than for PD (~30%); we did not find differences in activation for choice between games for either alpha or beta, in either ROI. This suggests choice between social dilemma games are no different on a neural level -- or rather, we use the same amount of activation for any social dilemma game even though the scenario or the payoffs are different. We also found differences in beta PFC for people who are cooperative as opposed to people who are competitive. People who are cooperative elicit greater beta PFC activation when cooperating than when defecting whereas people who are competitive do not show this difference. This can be related to risk assessment during the riskier choice of cooperation and also taking into consideration the future outcomes of a partner's choice. Further investigation into how robust this particular beta PFC finding is across social dilemma games can reveal support for PFC as an area related to risk assessment in social situations or risky decision scenarios.

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Poster

082. Social Decision Making

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Topic: F.01. Human Cognition and Behavior

Title: Distinct neuronal bases involved in the proposer and responder condition of the ultimatum game

Authors: *S. K. HORAT[1], G. FAVRE[1], F. R. HERRMANN[2], P. MISSONNIER[1,3], M. C. G. MERLO[1];
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Abstract: The behavior of humans in economic decision-making is well established in literature. The Ultimatum Game (UG) is a typical paradigm to investigate this issue. In this task, a "Proposer" has a certain sum of money at his disposal and has to propose a share to the "Responder", and the goal is to gain the maximum amount of money. The responder can either accept or reject this offer. If the responder accepts the proposal, the share is done accordingly whereas if he refuses, both players end up with nothing. However, the underlying cognitive processes are poorly studied yet. Therefore, we aimed to examine the neuronal bases of the proposer and responder condition performing event related potentials (ERP), independent component analysis (ICA) and source reconstruction. Two major ERP components, the P2 and feedback-related negativity (FRN) were observed in a time range of 150 to 400 ms after stimulus onset at medial electrode sites in both conditions. Although no significant differences were found for the FRN component, a significant shorter latency and increased amplitude of the P2 component for the proposer condition was observed. Moreover, ICA analysis showed a supplementary independent component activated in the P2 ERP time-range, modifying the P2 ERP morphology in proposer condition only. Additionally, the electrical source localization showed higher activity in the mid-frontal gyrus and anterior cingulate cortex for the proposer condition in the same time-range of the P2 ERP. Together, these results indicate an involvement of distinct neuronal bases at slightly different time points during decision-making suggesting that cognitive processes are engaged differently depending on the task condition although the goal is the same.

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Poster

082. Social Decision Making

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Topic: F.01. Human Cognition and Behavior

Title: A multi-variate pattern analysis investigation of strategic thinking and deception in a dynamic, competitive game

Authors: W. F. BRODERICK[1], R. M. CARTER[3], M. TEPPER[2], J.-F. GARIEPY[1], M. L. PLATT[1], G. SAPIRO[2], *S. A. HUETTEL[1];
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[3]Psychology, Univ. of Colorado, Boulder, CO

Abstract: Successful human interactions depend upon the interpretation of an agent's behaviors and intentions in order to support strategic control of behaviors and decisions. Interpreting others' actions requires many elements of social cognition, including more complex functions like theory of mind. Although there is some consistency in the neural substrates identified as supporting these functions (e.g. the medial-prefrontal cortex and temporal-parietal junction, TPJ), most tasks employ static or sequential interactions that do not allow investigation of differing levels of social-cognitive engagement on a short time scale. In this study, we adapted a dynamic, competitive game modeled on a simplified penalty kick, wherein humans (N = 29) controlled a ball, attempting to score against a human- or computer-controlled goalie, while measures of brain activation were obtained using functional magnetic resonance imaging (fMRI). This allowed us to quantitatively characterize subjects' strategic interactions with their opponent - and how these strategies change over short and longer time scales. K-means clustering on the difference between the y position of the subject-controlled ball and human-controlled goalie revealed that participant behavior reduced to two clusters, one pair representing early feints and misdirections (i.e., separation between participant and opponent near the start of the trial) and the other representing strategies intended to hide the kicker's intention for as long as possible (i.e., separation only at the end of the trial). These clusters - each further separable into pairs of upward or downward movements - correspond to distinct behavioral strategies. Play against a computer opponent served as a non-social baseline with similar strategic interactions. Using multivariate pattern analysis (MVPA), we examined whether local brain regions carried information that predicted trial features (e.g., opponent, outcome, strategy). Confirmatory analyses found that visual regions can be used to classify human from computer opponents at the time the opponent is revealed. MVPA of strategic interaction focused on the neural response differences between the two strategy-defined clusters. Using this dynamic task allows distinction of strategic elements of deception (e.g., timing of movement) from the conditions that elicit deception, the nature of the opponent, and the outcome of the action. The use of dynamic tasks facilitates the study of strategic social interaction on short timescales, opening avenues for causal studies of the supporting neural substrates.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIMH RO1 Grant MH087525

Title: Functional connectivity reveals network abnormalities during explicit and implicit moral reasoning in psychopathy

Authors: *K. J. YODER, J. DECETY;
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Abstract: Psychopathy is a strong risk factor for immoral behavior and is characterized by reduce empathy, remorse, guilt, and callous disregard for rights and welfare of others. It is also marked by abnormal attention which has downstream consequences on emotional processing. The current study used functional magnetic resonance imaging (fMRI) to examine the influence of task demands on moral evaluations in 88 incarcerated males (28 with Psychopathy Checklist-Revised (PCL-R) scores above 29). Participants viewed dynamic visual stimuli depicting interpersonal assistance and interpersonal harm in implicit and explicit moral reasoning tasks. In the first, psychopathy scores negatively predicted activity in striatum and dorsolateral prefrontal cortex, as well as functional connectivity seeded in right amygdala and right temporoparietal junction with anterior cingulate, anterior insula, striatum, and ventral prefrontal cortex. During the explicit task, psychopathy scores were negatively associated with response in anterior cingulate and amygdala. They also predicted decreased functional connectivity to striatum and temporal pole, but greater connectivity to dorsal anterior cingulate. These findings provide the first direct evidence for the role task demands play in the association between psychopathic traits and the salience network when viewing morally laden behaviors.

Disclosures: **K.J. Yoder:** None. **J. Decety:** None.

Poster

082. Social Decision Making

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Topic: F.01. Human Cognition and Behavior

Support: Res-062-23-3285

Title: Within-trial repetitive transcranial magnetic stimulation affects belief bias in conditional reasoning

Authors: *M. ROSER[1], J. S. T. EVANS[2], L. S. CARROLL[2], N. A. MCNAIR[3], G. FUGGETTA[4], A. KHARKO[2];
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Abstract: Human reasoning is affected by numerous biases. When asked to decide on the logical validity of conditional (if-then) statements, many people exhibit a bias towards endorsing inferences drawn from believable statements as logically valid. This is known as belief bias and it is hypothesized to involve neural systems for working memory and inhibition. To investigate the neural sources of belief bias we used brief within-trial repetitive transcranial magnetic stimulation (rTMS), guided by neuronavigation, to transiently disrupt activity in frontal brain regions identified as active in a large functional magnetic resonance imaging (fMRI) experiment of belief bias in conditional reasoning. Group fMRI results were warped into individual-subject space to identify cortical targets. The inferior frontal gyri (IFG, associated with inhibition) and dorsolateral prefrontal cortex (DLPFC, associated with working memory) bilaterally, and vertex, were targeted with 500ms of stimulation at 10Hz and 80% of active motor threshold at two separate stages (major premise and minor premise) of trials in which participants were instructed to conclude on the logical validity of conditional statements. Logical validity was crossed with the (independently-rated) believability of conditional statements in a design which has been shown to reliably produce belief bias. The experiment was run in six blocks of 24 trials with each block corresponding to stimulation of a different cortical site, the order of which was randomised. At the stage of Major-premise presentation, at which believability of the statement can be assessed, rTMS over the IFG increased belief bias relative to a sham condition. Stimulation of DLPFC at this timepoint did not affect belief bias. At the later stage of Minor-premise presentation, at which premises can be integrated and logical reasoning is possible, rTMS over IFG and DLPFC had no effect upon belief bias. These results suggest that belief bias arises as a result of insufficient inhibition of beliefs based on real-world knowledge at an early stage of the reasoning process.

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Poster

082. Social Decision Making

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Topic: F.01. Human Cognition and Behavior

Support: NUS R-581-000-123-133

NUS R-581-000-133-112

Title: Disgust sensitivity and pupillometric responses independently predict the effect of disgust stimuli on moral judgments

Authors: ***J. Z. LIM**[1], M. LEE[2], K. WONG[1], O. MULLETTE-GILLMAN[2,4,3];
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Abstract: Disgust stimuli alter moral judgment, but it is unclear if this modulation is due solely to disgust, or if physiological arousal may underlie this effect. Recently we demonstrated that disgust modulation of moral judgments across different domains is a bi-directional function of individual disgust sensitivity (DS). Here, we test whether arousal plays a mediating role in this relationship using pupillometry as a measure of state arousal. Participants (N=39) underwent pupillometry while judging the moral acceptability of utilitarian harm in personal moral dilemmas. Before each scenario, a disgust or neutral facial expression was presented for gender discrimination. Participants completed the Disgust Sensitive Scale (Revised) and the Affect Intensity Measure (AIM), a measure of trait arousability. First, we replicated that disgust sensitivity determines the degree and direction of the change in moral judgment, with significance in a one-way ANCOVA of change in ratings across disgust and neutral conditions with DS as a covariate ($F_{1,37} = 4.34$, $p = .04$, partial eta-squared = .11). We then examined whether arousal, as measured by pupillometry, could account for this modulation of moral judgment. We focused on a window 2-4s after the onset of facial primes; where prior studies have shown emotional stimuli modulate pupil dilation. We averaged the pupillometric difference between disgust and neutral conditions as a measure of the manipulated change in state arousal,

and examined whether this change could account for the change in moral judgment. This was accomplished through a multiple regression with both this arousal factor and individual DS as predictors of change in moral judgment. Both arousal and DS independently influence changes in judgment (Model R-squared = .195; DS: ß = 0.36, t = 2.50, p = .02; Pupil size difference: ß = 0.36, t = 2.46, p = .02). Our results show that both state arousal and disgust independently modulate moral judgments, and that arousal does not mediate the relationship reported in our first bidirectional function. This finding demonstrates that moral decision-making is not only the interplay of reason and emotion (disgust, moderated by individual sensitivity), but that additional physiological factors also play a critical role.

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Poster

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Title: Choking under pressure due to high incentives as a change in state distinct from motivated performance

Authors: *T. G. LEE, D. A. BARANY, S. T. GRAFTON;
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Abstract: One of the paradoxes in human motivation is performance failure that is seen in situations involving relatively large rewards or high motivational states. One strategy for understanding why a high reward could lead to performance failure (i.e. “choking under pressure”) is by mapping the underlying neural systems that are recruited during high pressure conditions. Using functional magnetic resonance imaging (fMRI), we recently found that activity in the dorsolateral prefrontal cortex and its functional connectivity with motor cortex is involved in modulating the extent to which participants choke under pressure during high incentive trials in a bimanual motor task. However it still remains an open question whether performance vulnerability under high incentive is due to a qualitatively separate “choking state” or is an effect

of a motor system that is 'overdosed' with too much arousal or other influence but otherwise functions the same as in low pressure states. By using a multi-voxel pattern analysis technique, we examined whether patterns of BOLD activity that are predictive of performance during a pre-movement preparatory period are similar across three different levels of incentive. Our results show that, across several brain regions including frontal and motor cortex, the activity patterns that lead to successful performance are similar at both low and medium levels of incentivized performance. In contrast, these patterns of activity fail to generalize in predicting performance at the highest level of incentive. This suggests that it is unlikely that choking under pressure simply reflects the higher end of a continuum of incentivized performance, but rather that performance vulnerability is due to a maladaptive state that is qualitatively distinct from usual performance situations.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: This study was supported by a grant from the Hilibrand Foundation.

Title: Predicting the preferences of others relies on self- and other-related prediction errors

Authors: *G. ROSENBLAU[1], C. KORN[2], B. VANDER WYK[1], K. PELPHREY[1];
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Abstract: Many of our efforts in social interactions are dedicated to optimizing our predictions of others' preferences, mental states, and behaviors, also referred to as Theory of Mind (ToM). Although much research has been devoted to identifying the neural implementation of ToM, it remains unclear how humans integrate self- and other-related information to predict others' preferences and how they optimize their predictions over time. Here, we utilize a novel preference task to investigate neural mechanisms involved in updating social predictions and learning from prediction errors. To ensure high ecological validity, the task involves real social feedback. Participants (N=21) are asked to predict the preferences of three different people for a number of items (food types, beauty products and leisure activities). They subsequently receive trial-by-trial feedback about the other's actual preference ratings. After completing the task,

participants rate their own preferences for the same items. On the single subject level, we modeled participant's trial-by-trial prediction errors (the absolute difference between participant's rating of the other person's preference and the person's actual preference rating) and the perceived self-other difference (the absolute difference between participant's ratings of their own preference versus that of the other person). Trial-by-trial computation of prediction errors and of perceived self-other differences was associated with activity in ventral and dorsal parts of the medial prefrontal cortex (MPFC). The computation of prediction errors versus perceived self-other differences elicited a stronger neural response in the dorsal part of the MPFC, superior temporal sulcus and in the ventral striatum. The opposite contrast yielded activity in the ventral MPFC and bilateral amygdala. We show that when participants infer another person's preferences, they encode the task-irrelevant difference between their own and the other person's preferences. Moreover, our results are in line with the notion of a dorsal-ventral functional gradient in the MPFC, distinguishing between other- versus self-relevant social processing. In a next step, we will apply computational learning models to delineate participants' individual social learning capacity by using past feedback to improve predictions for future items.

Disclosures: G. Rosenblau: None. **C. Korn:** None. **B. Vander Wyk:** None. **K. Pelphrey:** None.

Poster

082. Social Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 82.17/X5

Topic: F.01. Human Cognition and Behavior

Title: The use of strategy during social dilemmas: an EEG spectral analysis

Authors: *N. J. A. WAN[1], B. S. ROBINSON[1], A. J. WILSON[2], K. E. JORDAN[1];
[1]Psychology, Utah State Univ., Logan, UT; [2]Psychology, Western Illinois Univ., Macomb, IL

Abstract: The neural intricacies of strategy-driven choice within humans remain largely unknown. Using The Prisoner's Dilemma (PD), three types of strategies emerge: tit-for-tat (TFT), win-stay-lose-switch (WSLS), and no strategy. These strategies are also applicable in other social dilemma games, such as Snowdrift (SD). In this study, we investigate the behavioral and neural differences between TFT, WSLS, and no strategy across two social dilemma games, PD and SD. We expect to find people with strategies will perform better than people without strategies, regardless of the game. Neurally, we expect to find people with strategies to have

differences in prefrontal cortex (PFC; related to decision making) and/or inferior parietal lobule (IPL; related to social perception) activation. Lastly, we expect to find neural differences between two strategies, TFT and WSLS: TFT is a more socially-oriented strategy and should have increases in IPL versus PFC activation, whereas WSLS is more economically-oriented and should have increases in PFC vs IPL activation. We use EEG and analyze neural activation for alpha (8-12Hz) and beta (12-24Hz). Our results indicate greater behavioral scores for people with strategies as opposed to people without strategies, as predicted. Those with strategies have increased alpha desynchrony in IPL, suggesting the use of a strategy requires more neural resources than using no strategy. Those without strategy had differing activations by choice, in that cooperation showed greater alpha desynchrony than defection. Activation by choice was not different when choosing with a strategy. This suggests that processing a choice is more efficient for people with strategies, since activation for switching between choices is not different, whereas people without strategies have markedly different activation patterns for cooperation and defection, creating a larger difference in neural activation when switching between choices. Differences within strategies are notably found in the fronto-parietal network, where people choosing with TFT have increased beta PFC activation for cooperation as opposed to defection, whereas people choosing with WSLS show the opposite. Both strategies activate beta IPL greater than those without strategies. This PFC difference may indicate a relationship between economical decision making and social decision making: TFT can be framed as a more social-centric strategy, with increased beta for cooperation possibly indicating a theory-of-mind-type of assessment; WSLS can be framed as a more economical-centric strategy, with increased beta for defecting possibly indicating a cost-benefit-type of assessment.

Disclosures: **N.J.A. Wan:** None. **B.S. Robinson:** None. **A.J. Wilson:** None. **K.E. Jordan:** None.

Poster

082. Social Decision Making

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 82.18/X6

Topic: F.01. Human Cognition and Behavior

Support: BMBF Grant 01GQ1006

DFG Grant GRK1247

Title: Modeling social influence on human decision-making with reinforcement learning theory

Authors: *L. ZHANG, J. GLÄSCHER;
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Abstract: Most human decision-making takes place in a social context which influences individual decisions. In addition to making choices according to the action-outcome association, humans tend to align themselves with others, even without any direct social interaction. A long tradition of economic and social psychological studies has demonstrated a robust effect of social influence and conformity on perceptual decision-making (Asch, 1951). However, little is known about how the human brain computes value-based decisions when social influence is present. To fill this gap, we developed a novel experimental paradigm that allows for real-time interaction between multiple players. In the present study, groups of five participants were asked to perform a probabilistic reversal learning task, in which the reward probability switched after a certain number of trials. Following their initial choice, they were also required to place a post-decision wager between 1 and 3 (i.e., 1, 2 or 3) affecting their final payoff. After each participant had seen the choices from other four co-players via intranet, they were able to adjust both their choice and wager. In the end, monetary reward was displayed depending on the reward probability, and participants' goal was to maximize their payoff. Behaviorally, we observe an increased switch probability of choices as a function of group coherence levels (i.e., 2:2, 3:1 or 4:0) when their initial choice was in inconsistent with the collective decision of the others and an increased change in post-decision wagering along the group coherence when the initial choice was in agreement with the others. Using reinforcement learning, we built two "social" variants, in which the other players' choices could influence the subject's 2nd choice, and two "non-social" variants, in which subjects' 2nd choices were not affected by others. We employed Bayesian hierarchical estimation to fit these models to the behavioral data and used the deviance information criterion for model selection. The social models outperformed the non-social models. Between the social models, a variant that updates interim expected values by the weighted group coherence prior to the 2nd choice outperformed another model, in which the individual cumulative reward history was taken into account. Comparing the coherence model's action probability with the actual choice probability revealed that this model indeed provides robust prediction. These preliminary findings suggest that participants parse social information simply in terms of number of players (dis)agreeing with their initial choice rather than maintaining individual models of the others' decision-making process.

Disclosures: L. Zhang: None. **J. Gläscher:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 83.01/X7

Topic: F.02. Animal Cognition and Behavior

Support: R01 MH100822

Title: Astrocyte-neuronal lactate metabolism in memory formation during rat development

Authors: *E. CRUZ, A. TRAVAGLIA, C. M. ALBERINI;
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Abstract: Previous studies from our laboratory demonstrated that hippocampal astrocyte-neuronal lactate shuttling is critical for the consolidation of inhibitory avoidance (IA) memory in adult rats. Because the mechanisms underlying memory formation varies across age, and the transport of lactate from blood into the brain is higher during development, here we investigated the astrocyte-neuronal lactate metabolic mechanisms during memory consolidation across development. Specifically, we have focused on two developmental ages, postnatal day 17 (PN17), when the rats rapidly forget hippocampal-dependent memories (a phenomenon known as infantile amnesia), and PN24, when they are able to form strong and long lasting memories comparable to those of adult rats. We compared both groups to adult rats. We found that the hippocampal expression of lactate dehydrogenase A (LDHA), the enzyme that converts pyruvate into lactate, was significantly increased in total hippocampal extracts of both PN17 and PN24 rats compared to adult rats (PN80). Furthermore, the levels of hippocampal monocarboxylate transporter 4 (MCT4), a lactate transporter selectively expressed in astrocytes, and of MCT2, which is selectively expressed in neurons, were significantly lower in the synaptoneurosomal fraction of the P17 group compared to that of P24 and P80 rats. No differences were observed between the expression of the MCTs in P24 and P80. Furthermore, preliminary findings show that hippocampal infusions of the inhibitor of glycogen phosphorylase and synthase activity 1,4-dideoxy-,4-imino-d-arabinitol (DAB) at P24 impaired long-term memory, similar to the findings previously reported in adult rats. This data suggest that hippocampal lactate metabolism is developmentally regulated. This project was funded by NIMH R01 MH100822 to CMA

Disclosures: **E. Cruz:** None. **A. Travaglia:** None. **C.M. Alberini:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 83.02/X8

Topic: F.02. Animal Cognition and Behavior

Support: Gayle and Ben Batey Fund for the Neurosciences

CSUB Student Reseach Scholars Program

Title: High fat diets negatively impact female rodent learning and memory early in development and is counteracted by enriched environments

Authors: *I. C. SUMAYA, A. K. SUTER, S. HUSSAIN, N. RAMIREZ, C. DAWSON, S. MOMI, S. WILLIAMS;
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Abstract: The positive impact that enriched environments have on laboratory rodents has been well documented. Namely, rats kept in enriched environments have consistently shown improvements in learning and memory paradigms. Because the underlying mechanism of action of learning improvements is thought to be hippocampal neurogenesis, important to our research are the emerging data showing a reduction in neurogenesis in rats fed a high fat diet (Biotard et al., 2012; Valladolid et al., 2010). In line with these results, previously, we found high fat diets to negatively impact learning memory in male rats and used enriched environments to ameliorate these effects (Sumaya et al., 2014). Because there have been reports showing a high fat diet effects neurogenesis differently in male and female rats, we wanted to investigate the effects of a high fat diet on learning and memory in females. As we have done previously with males, we first placed female rats in either standard cages or enriched environments at 2 months of age and then fed them either Regular Chow (11% fat) or a Western Diet (39% fat, 44% carbs, 17% protein kcal, Modified AIN-93G, Research Diets) for 55 days then measured errors in the 8-arm radial maze at 4, and 10 months of age. Errors in the 8-arm maze task (averaged over the 8 days: 1 trial per day) were significantly greater in the high-fat group having its most detrimental effects in the groups that were housed in standard cages at 4 months of age (High Fat: 7.80±1.07 errors vs. Regular Chow: 3.13±0.63 errors). However, at 10 months of age, rats kept in standard cages and fed a high fat diet did not differ on learning errors as compared to their controls on regular chow. And, regardless of diet and age, the rats housed in enriched environments showed the least amount of errors at all testing points (Standard Chow at 4 months: 3.13±0.63 errors; 10 months: 1.44±0.41 errors) (High Fat at 4 months: 4.80±0.62 errors; 10 months: 2.20±0.50 errors). These data provide first time evidence that 1) increases in fat intake contributes to deficits in female rodent learning and memory at the early developmental stages, and 2) enriched environments can counteract the negative effects of a high fat diet on female rodent learning and memory.

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Poster

083. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 83.03/X9

Topic: F.02. Animal Cognition and Behavior

Title: Effects of enriched environment in persistence of social memory

Authors: *L. F. JAIMES, A. R. P. CAIXETA, A. F. S. ALMEIDA, G. S. PEREIRA;
Federal Univ. Minas Gerais, Belo Horizonte, Brazil

Abstract: Introduction: The enriched environment (EE) increase neurogenesis and improve performance of mice in many memories. Social memory is a capacity to recognize to co-specific and is crucial for reproduction, territorial defense and establishment of hierarchies in natural contexts. Previous studies in our laboratory demonstrated that 7 days of enrichment before training produce 10 days of persistence in social memory. However, other investigations proved that, after consolidation, neurogenesis induce by enrichment and pharmacological agents promotes forgetting in adult and young mice in a model of contextual fear. So our aim is determine effects of EE after a consolidation in a persistence of social recognition memory. Methods: Training session (TR) the Swiss juvenile male mice were used as intruder and were presented to the resident subject (with 30 minutes of habituation in the cage) inside a transparent acrylic cylinder during five minutes. The test session (TT) consisted in reintroducing the intruder juvenile mouse ten days after training to evaluate memory persistence. Adult male Swiss mice (8- to 12-weeks old) and juvenile male Swiss mice (25- to 30-days old) were maintained in two types of conditions with control of temperature and handling. Standard-housed (SH) consist in groups of five in a standard plastic cage. Enriched environment (EE) consist in groups of five in a larger plastic cage containing one plastic shelter, two cardboard rolls, three toys, and ribbons with distinct textures. Animals were divided into four experimental groups: EE+SH (7 days EE + TR + 10 days SH + TT), EE+EE (7 days EE + TR + 3 day SH + 7 days EE+ TT), SH+EE (7 days SH + TR+ 3 days SH+ 7 days EE+TT) and SH+SH (7 days SH+TR+ 10 days SH +TT). The social recognition index and social investigation time were analyzed by ANOVA and T-test when appropriated. Results: The groups with EE 7 days before training have persistence of social memory, so EE+EE (0.38±0.03) and EE+SH (0.36±0.03) remember the intruder, $p<0.05$. However, the EE after TT did not change the behavior of the animal on the task, then SH+EE (0.48±0.04) group did not remember the juvenile. Moreover, EE+EE did not show a statistical difference with EE+SH. Conclusions: This study could reproduce that 7 day of EE are enough to produce 10 day of social memory. However, EE after consolidation of social memory did not show influence in the remembering. Finnacial Support: CAPES, CNPq and FAPEMIG.

Disclosures: **L.F. Jaimes:** None. **A.R.P. Caixeta:** None. **A.F.S. Almeida:** None. **G.S. Pereira:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 83.04/X10

Topic: F.02. Animal Cognition and Behavior

Support: CNPq

Capes

Finep

Title: Age curve for memory expression: adult rats perform better than juvenile and middle-aged animals in the precision of fear conditioning context discrimination, Morris water maze and object location

Authors: ***A. P. CRESTANI**[1], L. DE OLIVEIRA ALVARES[2], J. HAUBRICH[2], F. SANTANA[2], R. O. SIERRA[2], J. QUILLFELDT[2];
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Abstract: The present study aims to investigate the performance of rats at three different ages in different types of memory. Specifically, we evaluated the precision of context discrimination in a fear conditioning task, Morris water maze learning and object location retention in juvenile (pubertal), adult and middle-aged rats. Our results show that animals at the two ends of the age curve retrieve memory traces with lower precision compared to adult animals. In the fear conditioning, adult animals were able to discriminate between the conditioned and the novel context when tested at 2 or 14, but not at 28 days after training: this replicates the classical trace generalization - with precision loss - that is characteristic of systems consolidation. However, juvenile animals could not discriminate contexts earlier, at 14 days, and middle-aged ones have lost this ability only 2 days after training. A similar “inverted U-shape” age-performance curve was observed in two tasks of a different nature, Morris watermaze and object location, where only adult animals were able to express adequate long-term retention. The converging results of three different, hippocampus-dependent memory tasks, suggests that memory deficits may not be restricted to aging animals - a classically established phenomenon - but is also a temporary

condition during ontogeny. To this point, these findings cannot be interpreted in terms of putative mechanisms, but it is reasonable to suppose that the qualitatively poorer memory of juveniles is due to some degree of immaturity of the involved brain areas, such as the hippocampus; a different mechanism would take place in the senescent brains, despite the similar performance observed. To our notice, this is the first study investigating differences of memory precision exhibited at distinct ages that not only encompasses juvenile animals in the full behavioral screening, but also found a deficit that is similar, but probably not related to the classical one observed in aging animals.

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Poster

083. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 83.05/X11

Topic: F.02. Animal Cognition and Behavior

Support: NSERC 341673

Title: Assessing the development of spatial memory consolidation in juvenile and adolescent rats

Authors: *N. TZAKIS, N. GILL, T. BOSNIC, M. R. HOLAHAN;
Carleton Univ., Ottawa, ON, Canada

Abstract: The neural mechanisms by which environmental stimuli are consolidated and stored as stable, long-lasting memory representations in the brain remain elusive. Investigations into the neural structures that mediate memory processing, and the development of connectivity patterns within those structures, have provided the foundation required to gain insight into the memory consolidation process. An understanding of how memories are stored, consolidated and used during the juvenile period will provide the next step to answering the fundamental questions concerning the transference of short-term memories into remote stores. Therefore, the main objective of the current work was to gain insight into the development of memory consolidation capabilities by examining and comparing memory consolidation processes between two developmental time points (juvenile and adolescence) based on the expression of spatial memories acquired either recently or remotely. Juvenile (P18) and adolescent (P50) rats were trained on the Morris water maze for 3 consecutive days and then tested either recently (24 hours after training) or remotely (3 weeks after training). The labeling of c-Fos was used as a marker

for neuronal activation in the hippocampus to assess the contribution of this brain region in the expression of the recent and remote memories. The juvenile and adolescent rats that underwent the probe test 24 hours after being trained spent about the same amount of time in the target quadrant, while the juveniles that were tested remotely spent significantly less time in the target quadrant compared to the other groups. The adolescent rats that were tested remotely spent significantly more time in the target quadrant showing the best memory. Additionally, c-Fos activation was significantly higher in the CA3 region of the hippocampus of the adolescent rats that were tested remotely compared to the other groups. These findings suggest that the acquisition and retention of spatial memories in rats vary relative to the degree of neural development, and that the processes involved in memory consolidation become more pronounced as the rat ages.

Disclosures: **N. Tzakis:** None. **N. Gill:** None. **T. Bosnic:** None. **M.R. Holahan:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 83.06/X12

Topic: F.02. Animal Cognition and Behavior

Support: CONACyT 155242

PAPIIT IN209413/24

Title: Differential consolidation of explicit and implicit memories under general anesthesia

Authors: *G. CHÁVEZ MARCHETTA[1,2], I. BALDERAS[2], C. J. RODRIGUEZ-ORTIZ[2], J. L. MCGAUGH[3], F. BERMUDEZ-RATTONI[2];
[1]Univ. Nacional Autónoma De México, Mexico City, Mexico; [2]IFC-UNAM, Mexico City, Mexico; [3]Ctr. for Neurobio. of Learning and Memory, Univ. of California, Irvine, CA

Abstract: Controversy exists whether memory formation can or cannot occur during anesthesia. In this work, we tested if different types of memory could be consolidated under general anesthesia. Pentobarbital was injected i.p. at a post-acquisition phase in rats in order to affect consolidation of different tasks; Morris water maze, Cued water maze, Object recognition, Conditioned taste aversion and Neophobic attenuation; each one related to different memory systems. We found that general anesthesia has different effects on memory consolidation; while implicit memory is consolidated, explicit memory is not. General anesthesia induced by

pentobarbital disrupted consolidation of Morris water maze, taste and object recognition memory and the extinction of conditioned taste aversion. However, memory consolidation for conditioned taste aversion and the cued Morris water maze was intact. These data indicate that pentobarbital disrupts the consolidation of explicit, but not implicit memories.

Disclosures: G. Chávez Marchetta: None. **I. Balderas:** None. **C.J. Rodriguez-Ortiz:** None. **J.L. McGaugh:** None. **F. Bermudez-Rattoni:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 83.07/X13

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

CFI

Title: Increased task demand during a spatial memory retention test recruits the anterior cingulate cortex

Authors: J. CARR, N. M. FOURNIER, *H. LEHMANN;
Psychology, Trent Univ., Peterborough, ON, Canada

Abstract: Spatial memory is believed to become increasingly dependent on the anterior cingulate cortex (ACC) over a protracted period. For instance, damaging the ACC in rats weeks after learning causes retrograde amnesia in the Morris Water Task, but the same damage just a few days after learning does not impair memory. Similarly, immediate early gene (IEG) expression in the ACC is greater for remote than recent spatial memories. These findings have been taken as evidence supporting systems consolidation, a process in which cortical structures, in this case the ACC, come to store memories that were initially dependent on the hippocampus. The recruitment of the ACC, however, can also be explained by an increase in cognitive demand when processing remote memories. This account does not imply a time-dependent storage progression, but a recruitment of the ACC because remote memories are more difficult to process. The current study tested this latter hypothesis by manipulating retention difficulty during a spatial memory test and assessing IEG expression in the ACC. Rats were trained in the hidden platform version of the Morris water task in a room rich in extra maze cues. The rats were then either tested for retention three days later in the same room with the same cue configuration

(Recent condition), three days later in the same room with prominent cues removed (Altered condition), or 30 days later in the same room with the same cue configuration (Remote condition). The intent with the cue removal in the Altered condition was to increase retention difficulty at time point that would have yet recruited the ACC because of systems consolidation. One hour after testing, the rats were euthanized, their brains sectioned, labelled for c-Fos, and quantified for c-Fos expression in the ACC using unbiased stereology. All three groups showed retention of the platform location during the test, but the performance was strongest in the Recent group. We also found, just like in other studies, that c-Fos expression in the ACC was significantly higher for the rats in the Remote condition than the Recent condition. Expression of c-Fos, however, was also significantly greater in the rats from the Altered condition than the Recent condition. Expression of c-Fos did not significantly differ between the Altered and Remote conditions. Thus, increasing cognitive demands and processing by reducing the number of cues available for retrieval during a recent memory test recruited the ACC similar to the extent of what is observed during remote memory testing. These findings do not preclude the involvement of the ACC in systems consolidation, but they provide evidence-based support for an alternative explanation.

Disclosures: J. Carr: None. **N.M. Fournier:** None. **H. Lehmann:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 83.08/X14

Topic: F.02. Animal Cognition and Behavior

Support: FAPESP

CNPq

CAPES

Title: Single bout of resistance exercise improves hippocampal memory consolidation

Authors: *J. C. SOARES[1], J. FERNANDES[2], L. G. Z. BALIEGO[2], R. M. ARIDA[2];
[1]Psychobiology Dept., [2]Physiol. Dept., Univ. Federal de São Paulo, Sao Paulo, Brazil

Abstract: Over the past decade, several studies have indicated that chronic resistance exercise (i.e., strength training, weight lifting, etc.) is beneficial for brain health and cognitive function. Human findings have demonstrated that regular resistance exercise can counteract the age-

related decline in cognitive function, ameliorate signs of Parkinson's disease, decrease anxiety and alleviate depressive symptoms. Over the last years, there has been a growing interest in the effects of exercise on changes in cognition after a single bout of acute resistance exercise. The idea that a single bout of resistance exercise can activate the molecular machinery needed for memory consolidation remains to be explored. Therefore, the purpose of the present study was to examine the effects of a single bout of resistance exercise applied immediately after the training of contextual fear conditioning on the long-term memory and on the expression of IGF-1 and synaptic proteins in the hippocampus. Methods: Male Wistar were familiarized with climbing a ladder (1.1 X 0.18 m, 2-cm grid, 80° incline) without load for three days and randomly assigned into Sham and resistance exercise groups. The resistance exercise group were submitted to a maximal carrying capacity test and 72 hours later subjected to a single bout of resistance exercise applied immediately after the fear conditioning training. Subsequently, animals were tested for contextual (24h) and tone (48h) fear memory. Another group of animals was submitted to a single bout of resistance exercise and 24 hours later were euthanized and the hippocampus were dissected to western blot and ELISA analysis of expression of IGF-1 and synaptic proteins (synapsin I, synaptophysin and PSD-95), respectively. Results: Exercised rats improved contextual ($p<0.05$) but not tone fear memory. Hippocampal IGF-1 expression was not altered by RE. However, the levels of synapsin I, synaptophysin and PSD-95 increased significantly in the hippocampus of resistance exercise group ($p<0.05$). Conclusion: Our results suggest that a single bout of resistance exercise applied immediately after the contextual fear conditioning training can improve hippocampal memory consolidation probably through activation of synaptic machinery.

Disclosures: J.C. Soares: None. **J. Fernandes:** None. **L.G.Z. Baliego:** None. **R.M. Arida:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 83.09/X15

Topic: F.02. Animal Cognition and Behavior

Title: Functional inactivation of hippocampus during memory consolidation of mixed choice task applied through the first learning trials is sufficient to suppress the use of hippocampal strategy for the subsequent trials

Authors: A. SERGEEVA[1], K. UZHCA[2], L. URPA[1], B. GROSS[1], *G. R. POE[3];
[1]Univ. of Michigan, Ann Arbor, MI; [2]New York Univ., New York, NY; [3]Dept Anesthesiol., Univ. Michigan, Ann Arbor, MI

Abstract: It has been widely accepted that hippocampal-based learning is the preferred learning strategy for the spatial food reward task in rats. Moreover, multiple factors have been associated with shifting the learning strategy toward alternative, non-hippocampal learning. However, no current research has explored how quickly animals restore use of the most efficient (hippocampal) strategy after withdrawal of hippocampus-suppressing treatment. To address this question, we trained animals on a mixed-choice food reward task using an eight-box octagonal maze. Long Evans rats were trained to search for food rewards hidden in constant positions relative to room cues. After each trial, the maze was randomly rotated, and the rat's starting position was changed randomly to reveal the contribution of different memory systems. Animals failing to use a hippocampal strategy remained able to find food every lap, but could only reduce the number of errors to 2 per lap. "Hippocampal learners" could reach 1 or fewer errors per lap after multiple sessions. Each group of rats was subjected to one of four treatments temporarily inhibiting hippocampal learning: (1) 2 hours of sleep deprivation, or (2) isoflurane anesthesia; (3) intrahippocampal norepinephrine infusion, or (4) optogenetic stimulation of the locus coeruleus during sleep. The treatment was applied following each training session during the offline consolidation period for the first three sessions. Post-treatment, animals were trained on the same task for the next 6 days/training sessions. The control group was subjected to the same procedures, but were allowed sleep ad libitum during periods when the experimental group was treated. All treatments induced suppression of the hippocampal strategy, not only during sessions following the treatment, but also for the next six days of training. The number of errors in the four groups was significantly higher than the control group. This suggests that treated animals did not shift back to the preferred/hippocampal strategy. To exclude permanent damage of the hippocampal memory system caused by treatment, animals injected with norepinephrine and optogenetically stimulated were re-trained on the same task in a different environment and maze. All rats were able to use the hippocampal strategy in a new environment and reached less than 1 error per lap by the end of the training, showing that treatment did not cause irreversible changes. Our findings indicate that functional inactivation of the hippocampus subsequently impairs cognitive flexibility. Dramatic changes in the environment of the task were required to restore the ability to update and optimize learning strategies.

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Poster

083. Memory Consolidation and Reconsolidation: Behavior

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Program#/Poster#: 83.10/X16

Topic: F.02. Animal Cognition and Behavior

Title: The amnesia induced by post-reactivation anisomycin-impaired reconsolidation of object location memory causes the brain to reengage the acquisition mechanisms of when there was no prior knowledge of that memory

Authors: *J. J. ZHANG, K. NADER;
Psychology, McGill Univ., Montreal, QC, Canada

Abstract: Experimental amnesia can be brought about by either impairing memory storage or memory retrieval. The traditional paradigm called recovery from amnesia cannot differentiate between whether the impairment is due to a storage or retrieval impairment. We previously developed a novel second learning paradigm that dissociates between the two (Hardt, Wang & Nader, 2009). This task depends on the finding that the first but not subsequent time animals acquire a memory, its acquisition is blocked by NMDAR-antagonist (2R)-amino-5-phosphonovaleric acid (AP5). To date, reconsolidation has been shown to occur in a variety of behavioural tasks including fear conditioning, contextual fear conditioning, incentive learning, to name a few. In contrast, few studies have explored reconsolidation of hippocampal-dependent spatial memory tasks such as object location. To this end, we identified the elements of the task required for reactivation of object location memory using four different open field contexts similar to the training context: with the bedding removed, with the objects removed, with objects removed and with different bedding, and with objects removed and with no bedding. Preliminary results revealed that reactivating the training memory with an open field with no objects, or no bedding and no objects, led to a reconsolidation effect. By establishing the elements required to induce reconsolidation we can then ask if reconsolidation blockade engaged first or second learning mechanisms by blocking reconsolidation of first learning memory, then testing for the susceptibility of second learning acquisition to AP5. In the next experiment, we induced reconsolidation of first learning object location memory, then trained the same animals with a second learning of object location paradigm. By blocking reconsolidation with bilateral infusions of anisomycin into the dHC, acquisition of second learning was AP5-sensitive. That is, second learning reverted to the AP5-sensitivity characteristically shown by first learning, not second learning acquisition. This suggests that blocking reconsolidation caused the second learning event to be treated as if it was the first time the object location information was acquired. Similar results have previously been attained with infusions of PKMζ-inhibitor ZIP after consolidation of first learning. We feel this task should be used more broadly to differentiate between storage or retrieval impairments.

Disclosures: **J.J. Zhang:** None. **K. Nader:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

Location: Hall A

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Marie Curie Intra-European Fellowship (FP7-PEOPLE-2013-IEF - 623638)

Title: The endocannabinoid system mediates stress-induced amnesia

Authors: *A. BUSQUETS-GARCIA[1,2], M. GOMIS-GONZÁLEZ[2], R. SRIVASTAVA[3], L. CUTANDO[2], A. ORTEGA-ALVARO[2], S. RUEHLE[3], F. REMMERS[3], L. BELLOCHIO[1], L. BINDILA[3], G. MARSICANO[1], B. LUTZ[3], R. MALDONADO[2], A. OZAITA[2];
[1]Neurocenter Magendie U862-Inserm, Bordeaux, France; [2]Laboratori de Neurofarmacologia. Departament de Ciències Experimentals i de la Salut. Univ. Pompeu Fabra, Barcelona, Spain; [3]Inst. of Physiological Chemistry, Univ. Med. Ctr. of the Johannes Gutenberg Univ. Mainz,, Mainz, Germany

Abstract: Memory consolidation is a labile process under the direct influence of emotional experiences. However, little is known about the underlying mechanisms. The endocannabinoid system plays an important role in the modulation of both emotion and memory, but the integration of these functions in the memory consolidation has not yet been addressed. This study investigates the involvement of cannabinoid type-1 (CB1) receptors in stress-induced amnesia for a non-emotional memory. Using a model for declarative memory in mice, the novel object-recognition test, we found that stress and the arousal state of the animal determine the consolidation outcome in this memory. Such a memory trace was obliterated under physical or psychological acute stress conditions. These amnesic effects of stress were not observed after local or systemic injection of the CB1 receptor antagonist rimonabant, and after the administration of the peripherally acting CB1 receptor antagonist AM6545 or genetic

inactivation of the CB1 receptor gen. According to these behavioral data, the c-Fos activation in different brain regions induced after shock was also blocked by the CB1 receptor antagonism. Using several cell type-specific conditional CB1 receptor knockout mouse lines, we found that CB1 receptors in dopamine β-hydroxylase (dbh) expressing adrenergic/noradrenergic cells are necessary and sufficient for the stress-induced amnesia, whereas CB1 receptors in other cell populations were not involved in this response. Notably, the removal of the adrenal glands prevented the amnesic-like effect of stress. In addition, the pharmacological modulation of adrenergic/noradrenergic system showed that both alpha- and beta-adrenergic receptors are involved in this stress-induced amnesia.In summary, central and peripheral adrenergic/noradrenergic transmission determine the consolidation of non-emotional memories, and this function is under the direct control of CB1 receptors expressed in dbh-positive cells. The elucidation of this mechanism opens novel therapeutic approaches for the treatment of memory- and stress-related disorders using drugs acting on peripheral CB1 cannabinoid receptors.

Disclosures: **A. Busquets-Garcia:** None. **M. Gomis-González:** None. **R. Srivastava:** None. **L. Cutando:** None. **A. Ortega-Alvaro:** None. **S. Ruehle:** None. **F. Remmers:** None. **L. Bellochio:** None. **L. Bindila:** None. **G. MArsicano:** None. **B. Lutz:** None. **R. Maldonado:** None. **A. Ozaita:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

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Support: 2015CB559200

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81271525

31300930

Title: Selective erasure of neural engrams of nicotine-associated memories to prevent nicotine craving and relapse

Authors: ***Y. XUE**[1], J. DENG[2,1], Y. CHEN[2,1], S. SUN[2,1], L. ZHANG[1], L. ZHANG[1], L. LU[2,1]; [1]Natl. Inst. on Drug Dependence, Peking Univ., Beijing, China; [2]Inst. of Mental Health/Peking Univ. Sixth Hosp. and Key Lab. of Mental Hlth., Beijing, China

Abstract: Background: Memories for substance abuse can be decreased by pharmacological interference after conditioned stimulus-induced memory reactivation (CS-MR). However, the procedure has limitations for “real world” relapse prevention, since the disruptive effect of the procedure is selective to the reactivated cues and does not generalize to other cues that have not been reactivated. In the present study, we investigated whether pharmacological interference after unconditioned stimulus-induced memory reactivation (US-MR) erases all nicotine-associated memory traces. Methods: In animal studies, rats were trained for pavlovian nicotine-associated memory with conditioned place preferences (CPP) and instrumental nicotine-associated memory with self-administration model. Memory was reactivated by exposure to previous nicotine-associated cues (as CS-MR) or a low dose of nicotine (as US-MR). In human study, smokers were trained for the association between pictures on the computer screen and smoking tobaccos. Memory reactivation was induced by smoking tobacco. Results: We found that cerebral ventricular microinjections of inhibitors of protein synthesis immediately after but not 9 h after CS-MR or US-MR disrupted subsequent recall of nicotine CPP memory. We also confirmed the phenomenon in instrumental nicotine-associated memory (nicotine self-administration). Subsequently, we found systemic administration of propranolol, an antagonist of adrenergic β-receptor, immediately after US-MR disrupted subsequent recall of nicotine CPP memory or nicotine self-administration memory. Furthermore, when rats were trained for two types of nicotine-associated memories (i.e. CPP memory and self-administration memory), systemic administration of propranolol immediately after US-MR disrupted subsequent recall of both of them, while propranolol administration immediately after CS-MR only disrupted subsequent recall of the memory reactivated previously. Lastly, after smokers learned the association between smoking tobaccos and two associated cues, oral administration of propranolol one hour before US-MR also decreased all the smoking-associated memories, including new learned cue memories and existing cue memories in real world. Conclusions: Our findings indicate that pharmacological interferences after US-MR lead to erasure of all nicotine-associated memory traces, and the US-MR-targeting inhibition may be a new strategy for the treatment of nicotine relapse.

Disclosures: **Y. Xue:** None. **J. Deng:** None. **Y. Chen:** None. **S. Sun:** None. **L. Zhang:** None. **L. Zhang:** None. **L. Lu:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

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Topic: F.02. Animal Cognition and Behavior

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Title: Histidine decarboxylase (HDC) and histaminergic receptors gene expression (H1, H2 and H3) acts distinctly along the timeline in the medial prefrontal cortex, dorsal hippocampus and amygdala after an aversive experience

Authors: *L. CANTO DE SOUZA[1], R. MATTIOLI[2];
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Abstract: Histamine acts as a modulatory neurotransmitter in the mammalian brain and there is evidence that it can modulate memory in different types of behavioral tasks. Here we investigated the HDC (histidine decarboxylase) and histaminergic receptors (H1, H2 and H3) gene expression in medial prefrontal cortex (mPFC), dorsal hippocampus and amygdala of male mice subjected to step-down inhibitory avoidance. The animals were assigned into 4 groups (n=4-5): control (naïve mice that did not experienced the aversive stimulus); 9 h post-training (mice that had their brains removed nine hours after training); 24 h post-training (mice that had their brains removed twenty four hours after training); 2 h post-test (mice that had their brains removed two hours after test). In mPFC we found an increase of HDC gene expression 24 h post-training and 2 h post-test; H2 gene expression increased 24 h post-training and 2 h post-test. In dorsal hippocampus HDC gene expression decreased 9 h post-training; 24 h post-training H2 and H3 gene expression increased; 2 h post-test H1 gene expression increased. Further, in amygdala, HDC gene expression increased 9 and 24 h after training; 9 h post-training H2 and H3 gene expression decreased and remained low after 24 h post-training and 2 h post-test; 2 h post-test H1 gene expression increased. We suggest that histamine acts distinctly along the timeline in the mPFC, dorsal hippocampus and amygdala after an aversive experience; dorsal hippocampus's H2 and H3 receptors and mPFC's H2 receptors might be involved in the aversive memory consolidation; dorsal hippocampus and amygdala's H1 receptor and mPFC's H2 receptors might be involved in emotional memory reconsolidation.

Disclosures: L. Canto De Souza: None. **R. Mattioli:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

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NCKU Aiming for the Top University and Elite Research Center Development Plan (MoE ATU Plan)

Title: A sex difference in the bidirectional effects of the CB1 antagonist/inverse agonist rimonabant on consolidation of cocaine-associated memory in mice

Authors: *S.-J. HU[1], H.-A. CHANG[2];
[2]Psychology, [1]Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Cannabinoid CB1 receptors are implicated in various forms of learning and memory, including cocaine-associated memory. Our previous study showed that systemic or intra-medial prefrontal cortex administration of the CB1 receptor antagonist/inverse agonist rimonabant impaired the consolidation of conditioned place preference (CPP) induced by a high dose (20 or 40 mg/kg) of cocaine, while facilitated that induced by a low dose (2.5, 5, or 10 mg/kg) in the male wild-type mice. These results suggest that rimonabant bidirectionally modulates the consolidation of cocaine-induced CPP memory. The current study aimed to investigate the receptor and sex hormone mechanisms underlying the bidirectional effects of rimonabant on cocaine-induced CPP. We first examined whether the CB1 receptor mediated rimonabant's effects by using the CB1 knockout mice and found that rimonabant did not facilitate memory consolidation of a low-dose (10 mg/kg) cocaine-induced CPP in both genders of the CB1 knockout mice. Likewise, rimonabant did not enhance the low-dose cocaine-induced CPP in the female wild-type mice. To test whether the sex hormone estrogen was involved, we tested rimonabant's effects on the low-dose cocaine-induced CPP in the vehicle-treated vs. estrogen-treated ovariectomized (OVX) female wild-type mice and found two interesting results. First, the estrogen replacement in the OVX mice per se enhanced the low-dose cocaine-induced CPP memory. Second, rimonabant facilitated cocaine-induced CPP memory in the OVX mice supplied with vehicle (sesame oil), while impaired the same memory in the OVX mice replaced with estrogen. Finally, rimonabant's effects on a high-dose (40 mg/kg) cocaine-induced CPP in both genders of the CB1 knockout mice as well as in the sham-control vs. OVX female wild-type mice are currently under investigation. Taken together, our results first indicate that the facilitating effect of rimonabant on the low-dose cocaine-induced CPP in the male wild-type mice is indeed mediated by the CB1 receptor. Second, estrogen is involved in the sex difference in the rimonabant's modulatory effects on cocaine-associated memory.

Disclosures: S. Hu: None. **H. Chang:** None.

Poster

083. Memory Consolidation and Reconsolidation: Behavior

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Topic: F.02. Animal Cognition and Behavior

Support: DA015222

Title: The naturally-occurring compound Garcinia indica impairs the reconsolidation of a cocaine-associated memory

Authors: *M. S. MONSEY, H. SANCHEZ, J. R. TAYLOR;
Mol. Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Sustained abstinence from cocaine use is frequently compromised by exposure to environmental stimuli that have previously been strongly associated with drug taking. Such cues trigger drug-associated memories, leading to craving and potential relapse. Our work has demonstrated that manipulating cocaine-cue memories by destabilizing them through interfering with the reconsolidation process is one potential therapeutic tool to prolong abstinence. For manipulations of established memories to be clinically useful, however, it would be optimal to identify a compound that can be administered systemically and that is natural with minimal side effects. We chose to assess the use of the naturally-occurring compound, garcinol, to manipulate an established cocaine-cue memory as it has previously been shown to block the reconsolidation of auditory Pavlovian fear memories in rats when given systemically (Maddox et al., 2013). Here, rats received 12 d of cocaine self-administration training during which time an active lever press resulted in a 1mg/kg i.v. infusion of cocaine while concurrently a cue light and tone were presented for 10 s. Next rats underwent lever extinction for 8 d, where lever pressing did not result in cocaine infusion or cue presentation. The following day, rats underwent memory reactivation in a novel context where the cues were briefly presented. Thirty minutes after reactivation rats received either a 10 mg/kg i.p. injection of garcinol or vehicle. Twenty-four hours after the reactivation session, rats were returned to the original chamber for a cue-induced reinstatement test where each active lever press resulted in a 10 s cue presentation. We found that post-reactivation garcinol is capable of impairing the reconsolidation of a cocaine-associated cue memory. These effects were also specific to a directly reactivated cue. Using non-reactivated controls we also show that the ability of systemic garcinol to effectively impair the reconsolidation of a cocaine-associated memory is predicated on active memory recall during the reactivation session; that is, in the absence of memory retrieval, garcinol has no effect on the retention of the memory. Additionally, we found that the effect of garcinol on reconsolidation is

temporally constrained; when rats are given systemic garcinol 6 h after reactivation there is no effect on retrieval. Therefore, systemic garcinol within a narrow window following memory retrieval can significantly impair reconsolidation. In sum, we show that systemic garcinol can block the reconsolidation of a cocaine-cue memory in a manner that is specific to the reactivated memory only and that is temporally constrained.

Disclosures: **M.S. Monsey:** None. **H. Sanchez:** None. **J.R. Taylor:** None.

Poster

084. Learning and Memory: Aging I

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

University of Florida Research Seed Opportunity Fund

1R21DA039701-01

Title: Age-related decline of spatial discrimination performance based on difficulty may reflect pattern separation deficits

Authors: ***S. A. JOHNSON**[1], L. S. GAYNOR[1], P. K. SACKS[1], S. M. TURNER[1], W. M. YODER[1], B. K. ORMEROD[1], A. P. MAURER[1,2], J. L. BIZON[1], S. N. BURKE[1,3]; [1]Dept. of Neurosci., Univ. of Florida, Gainesville, FL; [2]Biomed. Engin., Gainesville, FL; [3]Inst. on Aging, Gainesville, FL

Abstract: Cross-species evidence suggests that perceptual discrimination deficits are a core feature of age-related cognitive decline. In particular, individual aged rats impaired in spatial memory show correlated deficits in odor discrimination learning (LaSarge et al 2007 Neurobiol Aging 28:928). The goal of this study was to determine whether aging is associated with impairment of dentate gyrus (DG)-dependent perceptual discrimination ability in rats cross-characterized for spatial memory. Young (6-8 m) and aged (24-26 m) male Fischer 344 x Brown Norway rats were first trained on a spatial reference memory version of the water maze task (Bizon et al 2009 Neurobiol Aging 30:646). Relative to young, aged rats showed impaired spatial memory across probe tests [$p<0.04$]. Rats were then trained to perform a DG-dependent delayed-match-to-sample spatial discrimination task (Gilbert et al 2001 Hippocampus 11:626). In the task, rats learn to displace a target object covering a baited food well, and must recall the

location of this well based on peripheral cues when subsequently presented with two identical objects: one in the target location and one in a distractor location. Rats were initially trained to perform an easy version of the task, in which the distance between target and distractor objects was 80-95 cm. Young and aged rats learned this task within the same number of trials [p=0.21]. To assess spatial discrimination ability, rats were then tested on easy, intermediate, and difficult discrimination problems, in which the distances between target and distractor were 90, 48, or 15 cm, respectively. Young rats performed well above chance on both easy and intermediate problems [p's<0.001]. In contrast, aged rats performed above chance on easy [p0.24]. To further assess whether aging affects integration of spatial discrimination and memory functions, rats were trained to criterion and tested on intermediate problems with a delay of 1, 2 or 5 min imposed between cueing of the target location and choice between target and distractor locations. Recall of the target location was moderated by both delay duration [$F(5,70)=3.51$, $p<0.001$] and age [$F(1,14)=5.07$, $p<0.04$]. Considered together, these data suggest that age-related perceptual discrimination deficits may contribute to broader memory impairment. Furthermore, loss of spatial discrimination ability as a function of task difficulty is consistent with the interpretation that DG function is compromised by aging, and could reflect an underlying deficit in pattern separation computations performed by this region.

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Poster

084. Learning and Memory: Aging I

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1R21DA039701-01

Title: Nonlinear oscillations of the hippocampus

Authors: *A. P. MAURER[1], S. N. BURKE[1], A. SHEREMET[2];
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Abstract: Over 75 years of research have been dedicated to examining hippocampal oscillations in regards to their neurobehavioral correlates and mechanisms of generation (Jung and Kornmüller, 1938). The theta rhythm, which is 4-12 Hz fluctuation in the local field potential (LFP) prominent during movement and REM sleep (Vanderwolf, 1969), is perhaps one of the best described oscillations. Although early studies discussed theta as a nonlinear process (Leung, 1982), linear decompositions are often used to quantify the neurophysiological rhythms of the brain. Nonlinear measures, however, may be better suited for analyzing the dynamical processes in the nervous system (Aru et al., 2014). Therefore, in order to advance a fundamental understanding of the oscillations in the hippocampus, the current study utilized higher-order spectral analyses of the LFP data collected from the dorsal and intermediate regions of the hippocampus as rats traversed a circular track for food reward. A bispectral analysis was used to quantify the nonlinearities of theta, such as skew, asymmetry and the harmonics as a function of running velocity in both the dorsal and intermediate hippocampus. Moreover, the slope of the power spectral density was determined in both areas of the longitudinal axis. In the dorsal region, as movement velocity increased, the LFP became increasingly nonlinear showing higher skew, asymmetry and modulation of variance. Moreover, the bispectral analysis identified multiple harmonics of theta that developed as a function of running speed reaching a fifth harmonic at the fastest velocities. Finally, the power spectral properties of the LFP moved from pink- towards white-noise at faster running speeds. The emergence of nonlinear properties of the theta rhythm was also observed in the intermediate portion of the hippocampus, but the effect was attenuated. Together these data suggest that hippocampal LFP follows nonlinear dynamics that may reflect a dissipative system, which loses energy along the long axis.

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Poster

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McKnight Brain Research Foundation

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Title: Reductions in GABA(B) receptor signaling contribute to age-related impairments in behavioral flexibility

Authors: *S. BEAS[1], J. A. MCQUAIL[1], B. SETLOW[2], J. BIZON[1];
[1]Neurosci., [2]Psychiatry, Univ. of Florida McKnight Brain Inst., Gainesville, FL

Abstract: Behavioral flexibility involves the adaption of behavior in response to changing environmental demands, and this prefrontal cortex (PFC)-dependent executive function is critical for navigating complex environments. Loss of behavioral flexibility is associated with a number of neuropsychiatric diseases, and has been linked to inhibitory signaling dysfunction. Behavioral flexibility also significantly declines with age but the neurobiological causes of such decline are poorly understood. Previous work from our lab indicates that GABAergic signaling via GABA(B) receptors becomes dysregulated in the aged PFC. The current study combined molecular and pharmacological methods to determine if such age-related GABAergic signaling alterations contribute to impaired behavioral flexibility. Young adult (6 mo) and aged (24 mo) rats were tested on an attentional set-shifting task that assesses behavioral flexibility in which they were required to discriminate between two response levers to earn food rewards. Upon learning an initial response rule to criterion (e.g., visual cue = correct choice), the response rule was "shifted", such that the rat had to ignore the initial rule and respond according to a different rule (e.g., left lever = correct choice). Young and aged rats acquired the initial discrimination comparably, but aged rats were impaired relative to young on the set-shift. Notably, there was considerable variability among aged rats' performance, such that some performed within the range of young whereas others fell outside this range. Western blotting was then performed on homogenates prepared from micro-dissected medial PFC (mPFC) to determine expression of GABA(B)R1a and GABA(B)R1b subunits. Consistent with our previous work, expression of both subunits was significantly reduced in aged compared to young mPFC. Among aged rats, lower expression of both subunits was strongly associated with worse set shifting performance. These data suggest that age-related loss of mPFC GABA(B)R-mediated inhibition may contribute to impaired behavioral flexibility. To test this hypothesis, subsequent experiments determined whether the GABA(B)R agonist baclofen could improve behavioral flexibility in aged rats. Baclofen administered either systemically (2.5 mg/kg) or directly into the mPFC (0.5 nmol) in aged rats significantly enhanced performance compared to vehicle controls. Together, these data suggest that attenuated GABA(B)R signaling in mPFC contributes to age-related deficits in behavioral flexibility, and that GABA(B)Rs may represent a therapeutic target for remediating such deficits.

Disclosures: **S. Beas:** None. **J.A. Mcquail:** None. **B. Setlow:** None. **J. Bizon:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

University of Florida Research Seed Opportunity Fund

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Title: Age-related impairments in object-place associations signify a decline in systems-level neural communication

Authors: *J. E. REASOR[1], A. R. HERNANDEZ[1], S. M. TURNER[1], S. E. BATHLE[1], S. A. JOHNSON[1], A. P. MAURER[1,2], S. N. BURKE[1];
[1]Neurosci., McKnight Brain Institute, Univ. of Florida, Gainesville, FL; [2]Biomed. Engin., Gainesville, FL

Abstract: No single neurobiological deficit can account for the wide spectrum of cognitive changes observed in old age. In fact, most adaptive behaviors are supported by networks distributed across multiple brain regions, which renders the identification of a locus of dysfunction untenable. For example, episodic memory, which requires the integration of sensory stimuli with spatial locations, exhibits significant declines with senescence and relies on interactions between medial temporal lobe and neocortical structures. Therefore, understanding how different brain regions interact to support cognition is critical for uncovering the neurobiological mechanisms of age-associated cognitive decline. The neural circuit that supports memory includes the hippocampus (HPC) and the perirhinal cortex (PER). The HPC is critically involved in spatial reference memory (Morris et al., 1982), while the PER is necessary for object recognition (Winters et al., 2005). While both of these areas exhibit age-associated dysfunction (Rosenzweig and Barnes, 2003; Burke et al., 2010), deficits arising from the PER or HPC are often not correlated within individuals (Burke et al., 2010). Thus, it is unknown how aging impacts behaviors that require PER-HPC interactions. The goal of the current experiment was to determine the effect of aging on a task that requires communication between the HPC and PER. Young (4-6 months, n = 9) and aged (24-26 months, n = 14) rats were cross-characterized on a spatial reference memory version of the hippocampal-dependent water maze task and a PER-HPC inter-region dependent object-place paired association task (OPPA; Jo and Lee, 2010). Although aged rats did not exhibit a deficit in the spatial learning index or path length measures of water maze performance, ($F [1, 19] = 0.59$, $p = 0.45$), they were significantly impaired in

acquiring object-place associations relative to young ($F [1, 43] = 4.94$, $p < 0.05$). As this specific cohort of aged rats was unimpaired in spatial reference memory, these results demonstrate that deficits in the formation of object-place associations do not solely arise from generalized impairment of the hippocampus. Furthermore, these data support an emerging hypothesis that behaviors supported by functional connectivity across memory networks are particularly vulnerable to aging, as local dysfunction may propagate leading to global communication deficits across the memory circuit.

Disclosures: **J.E. Reasor:** None. **A.R. Hernandez:** None. **S.M. Turner:** None. **S.E. Bathle:** None. **S.A. Johnson:** None. **A.P. Maurer:** None. **S.N. Burke:** None.

Poster

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McKnight Brain Research Foundation

McKnight Brain Institute at the University of Florida

Title: NR2A-containing NMDARs in the PFC are required for working memory and predict age-related cognitive decline

Authors: *J. A. MCQUAIL[1], B. S. BEAS[1], K. L. SIMPSON[1], K. B. KELLY[2], C. J. FRAZIER[2], B. SETLOW[3], J. L. BIZON[1];
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Abstract: NMDA receptor (NMDAR)-dependent persistent firing of pyramidal neurons in the prefrontal cortex (PFC) is a likely neurophysiological correlate of working memory. NMDARs are tetrameric complexes comprised of obligate NR1 subunits that associate with NR2A or NR2B subunits to form a ligand gated ion channel. The subunit composition of the NMDAR complex confers unique channel properties and also determines retention at the synaptic zone versus diffusion to extrasynaptic sites. However, the contribution of specific subtypes of NMDARs to working memory and their changes with age are not well understood. To address these questions, the present study combined behavioral analysis of a delayed response (DR)

working memory task that is dependent upon an intact medial PFC (mPFC, the rodent homologue of dorsolateral PFC) and sensitive to aging, with behavioral pharmacological and molecular approaches in young adult (6 months) and aged (22-24 months) Fischer 344 rats. Experiment 1 evaluated behavioral consequences of acute intra-mPFC administration of NMDAR antagonists to young adult rats during DR task performance. Selective antagonists of NR2B-containing NMDARs, ifenprodil and Ro-25 6981, had no effect on DR task accuracy. In contrast, the NR2A-preferring antagonist NVP-AAM077 significantly impaired accuracy relative to vehicle. Experiment 2 used Western blotting methods to measure NMDAR subunit levels in mPFC homogenates prepared from a separate cohort of young adult and aged rats previously characterized on the DR task. NR1, NR2A and NR2B subunit expression was lower in aged compared to young rats, and NR2A protein expression was positively correlated with DR accuracy in aged rats. Apart from marginal reductions in AMPA receptor subunit levels, there were no other age-related changes in synaptic, spinous, or dendritic proteins. These experiments demonstrate that blockade of NR2A-NMDARs within the mPFC significantly impairs performance on a rodent working memory task. Moreover, attenuated expression of mPFC NMDARs coincides with onset of age-related working memory deficits and loss of NR2A subunit reliably predicts severity of working memory impairment. Jointly, these studies support a vital role for NR2A-NMDARs in working memory, possibly due to preferential localization at synaptic sites, and further suggest that their dysfunction may be a causal factor in working memory impairments that are prevalent among older individuals. Ongoing work will determine if positive modulation of synaptic NMDARs restores working memory in aged rats and evaluate age-related changes to NMDAR subunit:protein interactions.

Disclosures: **J.A. McQuail:** None. **B.S. Beas:** None. **K.L. Simpson:** None. **K.B. Kelly:** None. **C.J. Frazier:** None. **B. Setlow:** None. **J.L. Bizon:** None.

Poster

084. Learning and Memory: Aging I

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

University of Florida Research Seed Opportunity Fund

NIH Grant DA039701

Title: An open-source software suite for collecting and analyzing spontaneous object recognition data

Authors: *N. TOPPER[1], R. NDUM[1], A. R. HERNANDEZ[1], S. A. JOHNSON[1], J. REASOR[1], J.-M. MIZELL[1], S. TURNER[1], A. P. MAURER[1,2], S. N. BURKE[1,3];
[1]Neurosci., McKnight Brain Institute, Univ. of Florida, Gainesville, FL; [2]Biomed. Engin., Gainesville, FL; [3]Inst. on Aging, Gainesville, FL

Abstract: Behavioral neuroscience often necessitates tracking an animal as they move through an environment and quantifying exploration-related parameters. A common example is the spontaneous object recognition task in which the time a rat spends exploring an object is monitored (Ennanceur and Delacour, 1988). While there are several commercial options available for quantifying task parameters, purchasing software and multiple licenses may be cost-prohibitive for large-scale projects or budget-conscious laboratories. Moreover, without an open-source algorithm, end users are restricted to the structure of the original programming. Therefore, we have developed a two-part software suite that integrates with a web camera for data collection, organization and quantification. The first part of the code, dubbed "Collector", can load and process previously recorded data, or be integrated with a "live recording" for online tracking. Collector then tracks animal position, determines distance from user specified locations of interest (e.g., an area containing an object), and extracts threshold crossings. These variables are used in the second aspect of the software suite, "Minion", which provides an initial automated scoring of the behavioral data that can be refined by the user. For certain behavioral tests, such as spontaneous object recognition, exploration times are often scored when a rodent's nose is within a specified distance from an object. When complete autonomy is provided to tracking algorithms, there are often false positives in which the rat was in proximity, but not engaged with the object. Therefore, Minion provides a platform that allows user interaction and verification of exploration epochs provided by the algorithm. Exploration times can be calculated with the precision of the video framerate. Importantly, this scoring suite also offers the capability to automatically rename, and shuffle datasets, facilitating blind scoring (relative to serial analysis of video data which may have temporal cues to experimental conditions). Finally, the output of the algorithm, containing latency, exploration time, and length of each epoch of exploration, is readily converted to a comma-separated values file for import into Microsoft Excel and other downstream applications. In addition to spontaneous object recognition, this software suite can be used for animal tracking across a wide variety of behavioral paradigms. These include exploration or foraging in open arenas, tracking on land-based mazes (e.g. radial-arm, Barnes, and elevated-plus mazes), the Morris water maze, and monitoring of freezing behavior in fear conditioning paradigms.

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Poster

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NIH grant DA039701

Title: Object-place paired associations require interactions between prefrontal and perirhinal cortices

Authors: *A. R. HERNANDEZ[1], J. E. REASOR[1], S. M. TURNER[1], S. E. BARTHLE[1], S. A. JOHNSON[1], J. L. BIZON[1], A. P. MAURER[1,2], S. N. BURKE[1];
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Abstract: The networks that support episodic memory are distributed across the brain. Specifically, the ability to link a sensory stimulus with a spatial location, which is a cornerstone of episodic memory, requires communication between the medial temporal lobe and prefrontal cortex. In line with this idea, lesion studies have demonstrated that successful performance on the Object-Place Paired Association (OPPA) task requires the perirhinal cortex (PER), medial prefrontal cortex (mPFC) and hippocampus (HPC; Lee and Solivan, 2008; Jo and Lee, 2010a). This task tests an animal's ability to use their current spatial location to determine which of two objects is associated with a reward. While it is known that OPPA performance requires functional communication between the HPC and PER (Jo & Lee, 2010 J Neurosci 20:9850), it is unknown whether the mPFC acts in concert with these structures or functions independently to establish relevant goal representations. Importantly, mPFC activity has been shown to facilitate communication between PER and entorhinal cortices, which could promote the transfer of information between HPC and neocortex (Paz et al. 2007). To determine the role of PER-mPFC communication in forming object-place associations, young adult male Fischer 344 rats were trained on the OPPA task prior to receiving reversible disconnection lesions. After reaching criterion performance, rats were implanted with 4 cannulae bilaterally targeting the PER and mPFC. Following recovery and re-establishment of criterion performance, rats were tested 30 min after intra-cerebral infusions of the GABAA agonist muscimol or vehicle. Rats received, in a randomized sequence: 1) bilateral PER infusions, 2) bilateral mPFC infusions, 3) ipsilateral PER

and mPFC infusions, or 4) contralateral PER and mPFC infusions. Functional disconnection of PER-mPFC with contralateral muscimol infusions significantly impaired task performance, relative to vehicle infusions ($p<0.05$). Importantly, ipsilateral inactivation of PER and mPFC did not impair performance, such that rats performed at criterion levels ($p=0.57$). These results indicate that functional connectivity between the PER and mPFC is critical to the formation and referencing of object-location conjunctions. Together these data support the hypothesis that interactions between mPFC and rhinal cortices modulate HPC- PER functional connectivity. Thus, deficits in the OPPA task that occur in old age (see Reasor et al. 2015 SfN Abstract) may be better attributed to network dysfunction, rather than impairments within a single brain region.

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Poster

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McKnight Brain Research Foundation (JLB)

McKnight Brain Institute Post-doctoral Fellowship (JAM)

McKnight Pre-Doctoral Fellowship (CMH)

Title: Subregional and behaviorally-relevant transcriptional changes related to GABA and glutamate signaling in the aged rodent prefrontal cortex

Authors: *C. M. HERNANDEZ, III[1], J. A. MCQUAIL[1], B. S. BEAS[1], B. SETLOW[2], J. L. BIZON[1];
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Abstract: The rodent medial prefrontal cortex (mPFC) is considered the homologue to primate dorsolateral PFC and includes the prelimbic (PL) and infralimbic (IL) subregions. These subregions differ in their anatomical connections and functional contributions to a number of PFC-mediated behaviors. The normal aging process is associated with a decline in PFC-mediated executive functions such as working memory and behavioral flexibility, and previous work from

our laboratory indicates that dysregulated excitatory and inhibitory signaling within mPFC contributes to this decline. The goal of this study was to use targeted, low-density PCR arrays to extend these prior findings, and specifically to identify subregional and behaviorally-relevant age-related alterations in gene expression related to GABA and glutamate signaling. Young (6 mo) and aged (24 mo) F344 rats were first trained on mPFC-dependent set-shifting and delayed-response tasks that assess behavioral flexibility and working memory, respectively. Tissue punches (1 mm) from PL and IL were collected 2 weeks later for analysis of gene expression. There were surprisingly few differences in basal gene expression between the PL and IL subregions, and only a few robust changes were observed as a function of chronological age. Interestingly, however, several classes of genes were significantly upregulated in aged rats that performed comparably to young on the set-shifting and working memory tasks. These genes included subunits of ionotropic glutamate receptors, enzymes important for GABA/Glu synthesis, and excitatory and inhibitory amino acid transporters. Notably, the vast majority of these expression changes were only evident in the PL subregion. Together, these data indicate that gene transcription related to excitatory/inhibitory signaling can become markedly altered in the aged PFC, and, that excitatory/inhibitory signaling alterations in the PL subregion may be particularly relevant for executive function in aging.

Disclosures: C.M. Hernandez: None. **J.A. McQuail:** None. **B.S. Beas:** None. **B. Setlow:** None. **J.L. Bizon:** None.

Poster

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University of Florida McKnight Brain Institute Fellowship (to JAM)

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McKnight Brain Research Foundation (to JLB)

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Title: Chronic variable stress recapitulates age-related changes to glutamate and GABA receptors in the PFC

Authors: M. M. BRUNER[1], J. A. MCQUAIL[1], I. M. BACKES[2], R. R. CLIFTON[2], B. SETLOW[3], D. A. SCHEUER[2], *J. L. BIZON[1];
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Abstract: Normal aging is associated with impaired cognition, including executive functions supported by the prefrontal cortex (PFC). Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis also accompanies the aging process and a long-standing hypothesis in the cognitive aging field suggests that the cumulative effects of stress and concomitant glucocorticoid exposure over the lifespan precipitate neural changes that mediate the emergence of cognitive deficits. Our prior work strongly implicates altered glutamatergic and GABAergic signaling in age-related impairment of cognitive functions supported by the PFC. As this brain region is enriched in glucocorticoid receptors, the present study was designed to test the hypothesis that chronic, unpredictable stress alters expression of glutamate and GABA receptor subunits in a manner that mirrors the effects of advanced age. Young adult rats were exposed to a 14-day chronic variable stress (CVS) paradigm (an unpredictable schedule of stressors including insulin-induced hypoglycemia, 60 min restraint, 7 min swim in 16°C water, and predator urine) and brain tissue was collected on Day 15. Western blot analysis was used to measure protein levels of ionotropic glutamate receptor (NMDAR and AMPAR) and GABA(B)R subunits in the PFC of CVS rats and unstressed (UNS) controls (n=6-8/group). While NMDA receptor subunit expression (NR2A, NR2B) was not affected by stress, the AMPAR GluR2 subunit was significantly reduced in the CVS group (-29% vs UNS; $p<0.05$). In addition, CVS recapitulated a marked reduction in PFC GABA(B)R expression that is a consistent feature of aging. Expression of both GABA(B)R1a (-49% vs UNS; $p<0.05$) and GABA(B)R1b (-44% vs UNS; $p<0.05$) was significantly reduced in the CVS condition. This reduction in GABA(B)R subunits was not accompanied by a change in the expression of VGAT, a marker of GABAergic synapses. The finding of reduced AMPAR subunit expression, but preserved NMDAR subunit expression, supports morphological findings that CVS reduces stubby spine (AMPAR-enriched) density in the PFC but not thin spines (NMDAR-enriched). Furthermore, this study provides novel evidence that GABA(B)Rs in the PFC are a key player in the pathophysiology of stress-induced alterations to PFC function. Collectively, these data suggest substantial overlap in the molecular mechanisms through which psychogenic stress and aging impair PFC neural activity required for higher-order cognition.

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Poster

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Support: McKnight Brain Research Foundation

University of Florida Research Seed Opportunity Fund

NIH grant DA039701

Title: Cholinergic modulation of spatial discrimination performance in young and aged rats

Authors: L. S. GAYNOR[1], S. J. JOHNSON[1], P. K. SACKS[1], A. P. MAURER[1,2], J. L. BIZON[1], *S. N. BURKE[1,3];
[1]Neurosci., Univ. of Florida, Gainesville, FL; [2]Biomed. Engin., Gainesville, FL; [3]Inst. on Aging, Gainesville, FL

Abstract: Hippocampal-dependent mnemonic function is modulated by the neurotransmitter acetylcholine (ACh; Hasselmo 2006 Curr Opin Neurobiol 16:710; Parent and Baxter 2004 Learn Mem 11:9). It has specifically been proposed that elevated ACh levels during waking behavior and exposure to novelty serve to balance excitation and inhibition within the hippocampus, reducing interference to support perceptual discrimination function and memory consolidation. By consequence, ACh could play a significant role in regulating hippocampal-dependent computations, such as pattern separation and completion, which support perceptual discrimination. The goal of this study was to determine whether increasing ACh availability with the acetylcholinesterase inhibitor donepezil would alleviate age-related deficits in hippocampal-dependent spatial discrimination. Furthermore, we investigated whether ACh neurotransmission via alpha-7 subunit-containing nicotinic ACh receptors (nAChRs) is required for spatial discrimination performance. Young adult (6-8 m) and aged (26-28 m) male Fischer 344 x Brown Norway hybrid rats were trained to perform a delayed-match-to-sample spatial discrimination task (Gilbert et al 2001 Hippocampus 11:626; Johnson et al 2015 Society for Neuroscience abstract) and were tested to assess baseline discrimination ability. All rats were then tested following systemic injections of the cholinergic agonist donepezil (vehicle, 1 mg/kg, 3 mg/kg) and the alpha-7 containing nAChR antagonist methyllycaconitine (MLA; vehicle, 0.1 mg/kg, 0.5 mg/kg) using a within-subjects random blocks design. Drugs were injected intraperitoneally 40 min prior to testing on easy, intermediate, and difficult spatial discrimination problems. Donepezil improved spatial discrimination performance across both easy and intermediate problems in aged rats [$p<0.05$], but did not enhance performance beyond baseline in young rats [$p=0.37$]. In contrast, blockade of alpha-7 containing nAChRs with MLA impaired young rats' spatial discrimination ability. Considered together, these data implicate ACh neurotransmission in mediating hippocampal computations that support perceptual discrimination function across

the lifespan. Furthermore, these results suggest that increasing ACh levels through systemic treatment with donepezil is a viable therapeutic intervention for alleviating age-related cognitive decline.

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Poster

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McKnight Brain Research Foundation

Title: Aging alters excitatory and inhibitory modulation of GABAergic interneurons in layer 2/3 of the rodent medial prefrontal cortex

Authors: *K. B. KELLY[1], J. A. MCQUAIL[2], C. M. HERNANDEZ[2], J. L. BIZON[2], C. J. FRAZIER[1,2];
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Abstract: Recent animal studies suggest that age-related impairments in working memory are likely to be associated with increased inhibition of cortical pyramidal neurons in the medial prefrontal cortex. In the current study, we sought to extend our understanding of this phenomenon by more directly evaluating how aging alters the physiology and functional modulation of cortical GABAergic interneurons. Towards that end, we performed whole-cell patch clamp recordings from GABAergic interneurons in Layer 2/3 of the medial prefrontal cortex using slices extracted from both aged (20 month) and young adult (4 month) F344 rats. With respect to excitatory signaling, we found that interneurons, unlike pyramidal neurons, experience a loss of spontaneous excitatory input in age, and that the amount of current carried by NMDA receptors during an evoked EPSC is also reduced. Recent data further indicate that synaptic NMDA current in cortical interneurons in young animals is carried by combination of NR2A and NR2B containing receptors, and ongoing studies are expected to reveal whether NR2A or NR2B current is more severely impacted by age. With respect to inhibitory signaling, our results indicate that aging leads to a loss of tonic GABAergic inhibition experienced by

cortical interneurons, and that this change is likely associated with a functional increase in neuronal gain. These results also differ from, and likely compliment, prior observations made in pyramidal neurons. Future studies will attempt to evaluate whether specific subpopulations of cortical interneurons are particularly susceptible to tonic inhibition in young animals. Overall, our data reveal several clear and novel age related changes in the physiology and functional modulation of cortical interneurons, and provide additional mechanistic insight on how aging ultimately alters excitability in cortical networks that subserve executive function.

Disclosures: K.B. Kelly: None. **J.A. McQuail:** None. **C.M. Hernandez:** None. **J.L. Bizon:** None. **C.J. Frazier:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

University of Florida Research Seed Opportunity Fund

NIH grant DA039701

Title: A low-cost, open-source gait tracker for rodents

Authors: *R. NDUM[1], N. C. TOPPER[1], A. R. HERNANDEZ[1], S. N. BURKE[1,2], A. P. MAURER[1,3];
[1]Neurosci., McKnight Brain Institute, Univ. of Florida, Gainesville, FL; [2]Inst. on Aging, Gainesville, FL; [3]Biomed. Engin., Gainesville, FL

Abstract: Animal models of cerebellar disorders, Parkinson's disease, motor degeneration, arthritis and nerve regeneration often necessitate quantification of specific gait parameters in order to evaluate the efficacy of potential therapeutic interventions. Unfortunately, equipment for monitoring gait parameters is often cost-prohibitive and may reduce ethological validity if walking is restricted to a treadmill apparatus. Moreover, gait measurements that rely on this type of forced walking can be difficult to implement in rats. The current poster presents an open-source gait tracker capable of being used for rats and mice during natural movement as well as an algorithm capable of recording and quantifying gait variability, cadence and other parameters related to motor performance. Similar to other commercially available products, the present gait

maze operates on frustrated-total internal reflection trapping ultraviolet light within the acrylic until another medium (e.g., a paw) comes in contact with the floor. In other words, paw presses become illuminated proportional to the pressure of placement. The light is then recorded on a modified GoPro camera from Back-Bone Gear Inc. Specifically, the UV filter was removed, and the stock lens replaced with one of a variable focal length, in order to provide a low cost platform for recording at 120 framerates per second. Furthermore, an angled mirror is used to simultaneously record a profile view of the animal, allowing for the measurement of additional performance parameters, such as angle of spinal flexion and plantigrady. This implementation of a gait tracker can be assembled for less than $1500. Moreover, it does not rely on an enclosure for the subject, as is the case in commercially available products. Thus, this gait tracker is highly versatile and can readily be integrated with neurophysiological recordings, deep-brain stimulation, or other behavioral testing apparati to monitor gait in the context of a dual motor-cognitive task. Finally, providing initial open-source software, the intention of the current project is to make gait tracking readily available to laboratories at extremely low cost in order to accelerate collaboration, development and discovery.

Disclosures: R. Ndum: None. **N.C. Topper:** None. **A.R. Hernandez:** None. **S.N. Burke:** None. **A.P. Maurer:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NSF CAREER award 1256941

NRSA 1F31MH105161-01A1

Title: The effects of sleep deprivation on spatial representations in young and aged adult mice during the object-place recognition task

Authors: *R. K. YUAN, I. A. MUZZIO;
Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Sleep is thought to play a key role in memory consolidation, due to findings showing that neuronal ensembles activated during navigation are reactivated during sleep, and that sleep deprivation causes deficits in several hippocampus-dependent tasks. Moreover, previous

literature has shown that both cognition and sleep undergo several age-related changes. Here, we examined changes in sleep characteristics and cellular activity in young and aged adult C57bl/6 mice that were sleep-deprived after training in a hippocampus dependent object-place recognition task. On day 1, animals were habituated to a novel environment in which they subsequently explored an array of 3 objects placed in the environment, over the course of 3 consecutive training sessions. Immediately after the last training session, animals were sleep deprived for 5 hours using an automated sleep deprivation system consisting of a cylindrical enclosure with a continuously rotating bar. The following day, animals were reintroduced to the environment for a single test session in which one object was displaced. We found that aged animals and young sleep-deprived animals showed a reduced preference for the displaced object compared to young controls. Similar to previous findings in rats, young control mice displayed high place field stability from the third training session to the displaced object test session. However, aged animals and young sleep-deprived animals remapped strongly between the training session and the test session, suggesting that the animals perceived the context as novel. Additionally, we examined patterns of REM and NREM sleep following sleep deprivation and found that young sleep-deprived animals exhibited increased NREM sleep relative to controls, while aged animals showed increases in both REM and NREM. Our preliminary findings suggest that animals may need to maintain a stable representation of the context in order to perform the object-place recognition task, and that both sleep deprivation and age result in decreased place field stability in the long-term corresponding with impaired performance. Furthermore, sleep deprivation may have a differential effect on subsequent REM and NREM sleep in young and aged adult mice.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG016322

Title: Post-translational modifications of the NMDA receptor GluN2B subunit in the frontal cortex of old mice are related to spatial learning and cognitive flexibility

Authors: ***K. R. MAGNUSSON**, D. R. ZAMZOW, V. ELIAS, V. ACOSTA, E. ESCOBEDO; Dept Biomed Sci, Coll Vet Med. & Linus Pauling Inst., Oregon State Univ., Corvallis, OR

Abstract: The N-methyl-D-aspartate receptor (NMDAR) is particularly vulnerable to the effects of aging. The GluN2B subunit of the NMDAR, compared to other NMDAR subunits, suffers the greatest losses of expression in the aging brain, especially in the frontal cortex. One protein from the NMDAR complex, the scaffolding protein PSD-95, has an increased interaction with GluN2B in crude synaptosomes of the aged frontal cortex, which correlates with poorer learning. In this study we explored some of the mechanisms that may promote differences in the NMDAR complex in the frontal cortex of aged animals. Two ages of mice, 3 and 24 months, were behaviorally tested in the Morris water maze. The old mice were divided into two categories (good and poor learners), based on reference memory performance of young in place trials (threshold = mean + 2SD). The frontal cortex from each mouse was subjected to differential centrifugation, followed by solubilization in Triton X-100. Proteins from Triton insoluble membranes (synaptic membranes), Triton soluble membranes (extrasynaptic membranes), and intracellular membranes/cytosol were examined by Western blot. Although old mice designated as good learners performed worse than young, the old mice assessed as poor learners were significantly worse than both the young and good old in place learning. The old good learners showed impairments in reversal trials. Both old groups showed declines in GluN2B expression in the synaptic membranes of frontal cortex from young, with no change in PSD-95 expression. Levels of 115 kDa GluN2B cleavage product were found with greater intensity on Triton soluble membranes and GluN2B phosphotyrosine 1472 was increased in the synaptic membranes from frontal cortex of the old good learners, as compared to young. GluN2B phosphotyrosine 1336 was reduced and Fyn was increased in the old poor learners. These results suggest that better spatial learning in older individuals may negatively impact cognitive flexibility. The previously reported negative relationship of the PSD-95:GluN2B ratio in crude synaptosomes to memory may, in part, be due to good learners having more cleaved GluN2B in the extrasynaptic membrane than poorer learners. This may have been influenced by alterations in phosphorylation during aging.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Social memory of old-age mice and the effects of juvenile interactions

Authors: *A. S. ALMEIDA, A. R. P. CAIXETA, G. S. PEREIRA;
Inst. De Ciências Biológicas- Univ. Federal De Minas Gerais, Belo Horizonte, Brazil

Abstract: Introduction: Intuitively, we all know that aged people are usually very close to younger individuals. In fact, studies have showing that social stimulus can promote engagement in persons with dementia. Furthermore, social isolation accelerates, while social stimulus prevents the memory impairments observed in animal models for Alzheimer's disease. Here we tested the hypothesis that housing old-age mice with a juvenile mouse will decrease the cognitive impairments caused by natural aging and social isolation. Methods: Adult (2 months old) and old-aged (18 months old) male C57/BL6 mice and Swiss juvenile mice (21 days old) were used. Animals were maintained in 3 different housing conditions during one week: Adult mice housed with other adult mice (AAH), aged mice housed with other aged mice (OOH) and one aged mouse housed with one juvenile mouse (OJH). On the eighth day the animals were submitted to the social recognition paradigm to test social short- and long-term memories, S-STM and S-LTM, respectively. The social recognition index was analyzed by one-way ANOVA followed by the Bonferroni test. Results: S-STM was preserved in AAH (0.33±0.03) and OOH (0.36±0.04) groups, but impaired in OJH animals (0.54±0.05). Similar results were observed for S-LTM [AAH (0.37±0.01), OOH (0.36±0.02), OJH (0.63±0.06)]. Conclusion: Social memory was not impaired in old-age mice and the juvenile stimulus impaired their social memory, which was the opposite of what we predicted. We intend to evaluate other types of memories and also to verify if long-periods of co-housing old-age and juvenile mice would present a positive effect on cognition. Financial support: FAPEMIG, CNPq and CAPES.

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Topic: F.02. Animal Cognition and Behavior

Support: AFAR New Investigator Grant RAG14141

Title: Contextual fear memory is impaired in middle-aged breeders vs non-breeders

Authors: *L. A. WILMOTT[1,3], S. M. NEUNER[1,3], T. M. SHAPAKER[1], R. W. WILLIAMS[2,3], C. C. KACZOROWSKI[1,3];

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Abstract: Over 80% of women will experience pregnancy and childbirth, and it is well known that both the brain and behavior are altered with pregnancy. Studies have found that rats that have more than one litter (multiparous) exhibit better reference and working memory and less hippocampal neurodegeneration with age (Kinsley et at, 1999; Pawluski et al, 2005; Gatewood et al, 2005); however, humans exhibit a decline in verbal recall memory that lasts from pregnancy through 3 months postpartum (Glynn, 2012). Studies involving the effects of pregnancy and reproductive experience on learning in mice are lacking. Based on the previous rat literature, we hypothesized that multiparous female mice would exhibit enhanced memory on a hippocampal dependent contextual fear memory task. To analyze this, we employed both multiparous and virgin (nulliparous) females from the BXD mouse panel (n=10 strains/grp), a genetic reference panel in which the resulting lines are descended from crosses between C57BL/6J and DBA/2J and are 99.5% isogenic yet genetically diverse. We found that reproductive experience significantly affected contextual fear memory in middle aged (12-20 mo) female BXD mice. Specifically, middle-aged multiparous BXD mice had significantly worse contextual fear memory compared to their nulliparous counterparts (paired two-tailed t-test: p=0.002). These results suggest that reproductive experience in middle-aged BXD mice negatively affects hippocampal function. Follow up studies using the BXD reference panel, which have been densely phenotyped and provide an excellent resource to study both genetic and phenotypic variation across a population in traits, will enable dissection of genetic mechanisms on cognitive functions and other phenotypic traits related to human mental health, including depression and anxiety. Identification of novel gene variants and/or unexpected related health traits that compound or mitigate the memory decline following childbirth is of major significance for biomarker development and personalized therapeutics.

Disclosures: L.A. Wilmott: None. **S.M. Neuner:** None. **T.M. Shapaker:** None. **R.W. Williams:** None. **C.C. Kaczorowski:** None.

Poster

084. Learning and Memory: Aging I

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 84.17/X38

Topic: F.02. Animal Cognition and Behavior

Title: Nest building and circadian rhythm activity is impaired in APOE4 mice compared to C57 mice

Authors: *K. BOGGS[1], K. A. PEDEMONTE[1], C. L. C. NEELY[1], S. A. STAVROU[1], S. N. HOWELL[1], L. BOZZELLI[2], J. M. FLINN[1];
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Abstract: Nest building by mice is an innate behavior that provides the animal with shelter and temperature regulation. Because nest building is instinctive for mice, it is often used to assess animal welfare. The ability of mice to build nests is influenced by several factors including temperature and illness, and changes in nesting suggest an alteration to the environment or the animal itself (Gaskill et al., 2013). Hess et al. suggest that nest building is additionally influenced by the biological relevance of the nesting material. Previous research on nest building in C57 mice has reported significant nest building results using pressed cotton squares (Deacon, 2006). However, attempts by our lab have been made using cotton squares with Alzheimer's (AD) mouse models generated on a C57 background, with variable results. Many AD patients have deficits in activities of daily living (ADL), including activities such as bathing, dressing, and feeding, and nest building serves as a measure of ADL in mice. It is essential to establish an appropriate nesting material in genetically modified mice before conducting an experiment, to ensure that nesting behavior is a result of the animal model rather than the material provided. Thus, 4 different nesting materials were tested in C57 (n=10) and APOE4 mice (n=11), older than 9 months. The nesting materials included iso-BLOX cotton squares (Harlan 6060), Diamond Twists (Harlan 7979C.CS), Soft Cob bedding (Harlan 7987C), and shredded paper. To assess nest building, animals were individually housed for 2 days with each of the 4 nesting materials, for 4 nesting trials per animal. Results indicate that shredded paper is more reliable than the other 3 nesting materials ($p<0.01$), followed by Soft Cob bedding, which was significantly better than the other 2 nesting materials ($p<0.05$). There was no significant difference between iso-BLOX cotton squares and Diamond Twists. Additionally, there was a main effect of genotype, with the E4s performing worse ($p<0.01$). Another aspect of daily living is circadian rhythm (CR) activity, which is measured in mice by observing wheel running activity. In a 5-day CR assay, C57s consistently initiated activity at 19:00 hours, while E4s demonstrated a delay in activity onset by 6 hours for 2 of the testing days ($p<0.05$). C57s had longer bouts and more counts/bout, suggesting that E4s had more random circadian rhythm activity. A day-by-day repeated-measures ANOVA indicated a day*genotype interaction ($p=0.05$) for number of bouts/day. While number of bouts for E4s were shorter in duration and of fewer counts, bouts/day were significantly higher ($p<0.05$), demonstrating a significant main effect of genotype.

Disclosures: K. Boggs: None. **K.A. Pedemonte:** None. **C.L.C. Neely:** None. **S.A. Stavrou:** None. **S.N. Howell:** None. **L. Bozzelli:** None. **J.M. Flinn:** None.

Poster

084. Learning and Memory: Aging I

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Topic: F.02. Animal Cognition and Behavior

Support: AG38747

NS56218

Title: Disparate brain lipid profiles in mouse models of IGF-1 deficiency

Authors: *S. LOGAN[1], J. E. SANDERS[1], N. M. ASHPOLE[1], R. S. BRUSH[2], R. E. ANDERSON[2], W. E. SONNTAG[1];
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Abstract: Insulin-like growth factor-1 (IGF-1) deficiency with age is associated with impairments in learning and memory. Nevertheless, different models of IGF-1 deficiency (e.g. the growth hormone receptor (GHR) and IGF-1 knockout) appear to have diverse effects on cognitive function. Previous studies indicate that growth hormone (GH) can regulate hepatic lipid biosynthesis but the effects on cognitive function are unknown. Lipids have been shown to mediate critical aspects of neuronal function. Changes in the lipid milieu in brain have been reported in aging and age-related neurodegenerative diseases. In this study, we performed a comparative analysis of the brain lipid profile in two models of IGF-1 deficiency. Our hypothesis is that models of systemic GH/IGF-1 deficiency have unique effects on brain lipid profiles and cognitive function. We induced knockdown of 1) the hepatic growth hormone receptor (GHR) and 2) hepatic IGF-1 using liver-specific AAV8-TBG-Cre (L-Cre) or -GFP (L-GFP). Mice, either growth hormone receptor floxed (GHRf/f) or IGF-1f/f, were retro-orbitally injected with AAV8 at 2-3 months of age. Mice were assessed behaviorally at different timepoints post-injection using the radial arm water maze (RAWM) for spatial memory. Following behavioral testing, tissues were harvested and brain regions were analyzed for lipid profiles. GHRf/f-L-Cre mice (6m post knockdown) showed deficiency in the reversal task in behavioral analysis indicating a deficit in memory compared to -L-GFP controls. IGF-1f/f-L-Cre animals (3m and 1y post knockdown) showed no behavioral differences compared to -L-GFP controls. In both animal models Cre-injected animals showed ~60% reduction in circulating IGF-1 levels compared to GFP controls. GHRf/f-L-Cre mice showed significant changes in hippocampal phospholipids compared to GHRf/f-L-GFP. MUFA synthesis (c18:1) was increased in the

hippocampus as well as in the serum of the GHRf/f-L-Cre mice compared to -GFP controls. IGF-1f/f-L-Cre mice, with comparable circulating IGF-1 levels to the GHRf/f-L-Cre mice, showed no significant changes in hippocampal phospholipids profiles or fatty acid synthesis. While there may be a compensatory mechanism in the brain of IGF-1f/f-L-Cre mice regulating fatty acid synthesis, the lipid changes in the GHRf/f-L-Cre mice suggest an effect of growth hormone in fatty acid/lipid regulation, which is correlated with deficiency in cognition. By understanding the specific role of these lipids in brain function and its regulation by IGF-1, we expect to develop metabolic targets of therapeutic potential to avert or delay age-related cognitive impairment.

Disclosures: S. Logan: None. **J.E. Sanders:** None. **N.M. Ashpole:** None. **R.S. Brush:** None. **R.E. Anderson:** None. **W.E. Sonntag:** None.

Poster

084. Learning and Memory: Aging I

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Topic: F.02. Animal Cognition and Behavior

Support: AG38747

Title: Early life IGF-1 deficiency results in age-related cognitive impairment

Authors: E. L. HODGES[1], N. M. ASHPOLE[2], *W. E. SONNTAG[2];
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Abstract: Previous studies indicate that the decline in circulating IGF-1 with age contributes to cognitive dysfunction. Nevertheless, studies have reported differential cognitive outcomes of young animals deficient in hepatic IGF-1. To address these disparate effects, we developed mouse models in which a deficiency of circulating IGF-1 can be initiated at different stages of the lifespan. Here, we report the behavioral results of a comprehensive study on the time-dependent effects of IGF-1 deficiency on lifespan and behavior in C57BL/6 mice. Cognitive assessment was performed on male mice with either a hepatic specific knockout of IGF-1 (induced at p15) by crossing $Igf^{f/f}$ mice with albumin-Cre or $Igf^{f/f}$ mice injected with a hepatic specific AAV-Cre (AAV8-TBG-Cre) or AAV8-TBG-eGFP at 5 or 15 months of age. In each group, hepatic IGF-1 knockdown reduced serum IGF-1 levels by at least 50% compared to controls. Using the Barnes maze to assess spatial learning and memory, we find that male mice deficient in circulating IGF-1 beginning at p15 and tested at 26 months of age are cognitively

impaired when compared to non-deficient litter-mate controls. During probe trials, performed 24 hours after the last day of after acquisition, animals with early onset IGF-1 deficiency (p15) were only able to find the platform 17% of the time compared to 58% in their litter-mate controls. Additionally, these animals visited the target location less frequently than control groups. These deficiencies were not observed when circulating IGF-1 was reduced at 5 or 15 months of age. Our results provide compelling evidence that the age of onset of IGF-1 deficiency has an important influence on the development of cognitive dysfunction in C57BL/6 mice.

Disclosures: E.L. Hodges: None. **N.M. Ashpole:** None. **W.E. Sonntag:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

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Program#/Poster#: 85.01/X41

Topic: F.02. Animal Cognition and Behavior

Support: NIMH R01 MH060013

NIMH R01 MH061492

Title: A novel slow (1-3 Hz) oscillatory cell type in the lateral septum

Authors: *J. R. HINMAN, J. R. CLIMER, G. W. CHAPMAN, M. E. HASSELMO;
Ctr. for Memory and Brain, Boston Univ., Boston, MA

Abstract: A multitude of oscillatory patterns are present in the brain ranging in cycle duration from hours to seconds to milliseconds. Varying constellations of channels endow individual neurons with the capability to oscillate at these various frequencies. We performed multiple single unit recordings in awake behaving rats in both the medial septum (MS) and the adjacent lateral septum (LS), which is distinct from its medial neighbor based on the neurochemical composition of its constituent neurons and its afferent and efferent projections. The oscillatory properties of neurons in the two regions were compared. A novel oscillatory cell type was identified in LS with a frequency ranging from 1 to 3 Hz. Unlike classically described delta frequency activity, the slow rhythmic firing of these LS neurons does not occur during slow wave sleep. In fact, the oscillatory activity is most robust during locomotor behavior during which the oscillation frequency increases as a function of running speed, similar to the manner in which theta rhythmic neurons in MS have oscillation frequencies that increase as a function of running speed. Slow rhythmic LS neurons are not simply firing on an interval of multiple theta

cycles, as is observed in neurons that fire on alternate theta cycles. There is actually a lack of any relationship between slow rhythmic LS and theta rhythmic MS neurons. Cross-correlations between simultaneously recorded slow rhythmic LS neurons and theta rhythmic MS neurons revealed no relationship between the two oscillatory cell types and slow rhythmic cells have no theta phase preference. Despite the lack of temporal coordination between the two oscillatory cell types, both slow rhythmic LS neurons and theta rhythmic MS neurons have strong temporal interactions amongst themselves. Both oscillatory cell types maintain a variety of phase relationships with other oscillatory cells of the same frequency. Overall, slow rhythmic LS neurons behave similarly to theta rhythmic MS neurons, albeit at a slower frequency, and may play a role in updating or synchronizing downstream supramammillary nucleus circuits.

Disclosures: J.R. Hinman: None. **J.R. Climer:** None. **G.W. Chapman:** None. **M.E. Hasselmo:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

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Program#/Poster#: 85.02/X42

Topic: F.02. Animal Cognition and Behavior

Support: NIMH R01 MH60013

NIMH R01 MH61492

ONR MURI N00014-10-1-0936

Title: Medial septal infusion of a serotonin 1A receptor agonist anxiolytic reduces theta frequency in the medial entorhinal cortex

Authors: *C. MONAGHAN[1,2,3], G. CHAPMAN, IV[1,3], M. HASSELMO[1,3];
[1]Dept. of Psychological and Brain Sci., [2]Grad. Program for Neurosci., [3]Ctr. for Systems Neurosci., Boston Univ., Boston, MA

Abstract: Theta rhythm is a prominent 6-10 Hz oscillation that can be found in the local field potential (LFP) and rhythmicity of single unit firing throughout many areas of the rodent brain, including the medial septum (MS), hippocampus, and medial entorhinal cortex (MEC). This rhythm can be disrupted through interfering with normal activity within the MS with lesions (Mitchell et al., 1982) or pharmacological inactivation (Mizumori et al., 1990). Temporarily inactivating the MS pharmacologically also disrupts the spatially periodic firing of grid cells

recorded in the MEC (Brandon et al., 2011; Koenig et al., 2011). Anxiolytics provide a method of investigating mechanisms underlying grid cell firing and their associated circuit, as anxiolytics have been shown to reduce the frequency of theta rhythm recorded in the hippocampus (McNaughton et al., 1986; Coop & McNaughton, 1991; Wells et al., 2013) and the mEC (Monaghan et al., 2014, Soc. Neurosci. Abstr.). While multiple types of anxiolytic drugs reduce the y-intercept, and not the slope, of the relationship between theta frequency and running speed when given systemically, it is not known if effects are exerted through actions on the MS. Here we report the effects of MS infusions of the serotonin 1A receptor agonist 8-OH-DPAT and the benzodiazepine diazepam on LFP activity and firing properties of single units recorded in the MEC. We found that MS infusions of 8-OH-DPAT, but not diazepam, reduced the y-intercept of theta frequency across running speeds, suggesting that benzodiazepines decrease theta frequency when given systemically via actions in a different brain region. This result lends support to the prediction made by John et al. (2014) that benzodiazepine action in the septo-hippocampal circuit is not sufficient to produce significant effects on theta frequency, which can perhaps be extended to the septo-entorhinal circuit as well.

Disclosures: C. Monaghan: None. **G. Chapman:** None. **M. Hasselmo:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

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Program#/Poster#: 85.03/X43

Topic: F.02. Animal Cognition and Behavior

Support: NIMH R01 MH61492

NIMH R01 MH60013

ONR MURI N00014-10-1-0936

Title: *In vivo* rebound spike characteristics of medial entorhinal cortex cells

Authors: *Y. TSUNO[1,2], G. CHAPMAN[2], M. E. HASSELMO[2];
[1]Psychology, [2]Ctr. for Memory and Brain, Dept. of Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: Medial entorhinal cortex is an anatomical gateway between neocortex and hippocampus, and plays a critical role in spatial learning and memory. The medial entorhinal cortex contains a variety of spatially modulated cells, including grid cells. Grid cells have

attracted interest because of their highly-organized hexagonal response pattern indicating an allocentric representation of the outside world. On the other hand, medial entorhinal cortex layer II stellate cells predominantly interact via inhibitory feedback, and it is possible that hyperpolarizing input affects the probability of subsequent spikes due to rebound potentials and rebound spikes. Our laboratory recently proposed a model incorporating rebound spikes to generate grid cell activity. To examine the characteristics of rebound spikes *in vivo*, patch clamp recording was performed from medial entorhinal cortex cells in ketamine and xylazine anesthetized mice. We injected brief hyperpolarizing current at various phases of the oscillation superimposed with sinusoidal oscillation, and found that hyperpolarizing current stimulation at specific phases of sinusoidal oscillation increased the probability of subsequent spikes at the peak of the oscillation in some cells. Also, the effect of the hyperpolarizing input to subsequent spikes is larger in the cells that had larger sag amplitude, indicating a relationship between sag potential and rebound spikes. In addition, larger and longer hyperpolarizing current square pulse stimulation could result in larger probability of eliciting rebound spikes. We did not observe a relationship between amplitude or duration of hyperpolarizing current square pulse stimulation and the temporal delay for rebound spikes relative to the end of hyperpolarizing current stimulation. These results suggest that rebound spikes exist *in vivo* and may play a critical role for emerging grid cell activity in medial entorhinal cortex cells.

Disclosures: **Y. Tsuno:** None. **G. Chapman:** None. **M.E. Hasselmo:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

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NIMH Grant 1F31MH102022-01A1

ONR MURI award N00014-10-1-0936

Title: Optogenetic silencing of septal cholinergic cells, memory and hippocampal theta

Authors: *J. R. CLIMER[1,2], M. E. HASSELMO[1];
[1]Dept. of Psychological and Brain Sci., [2]Grad. Program for Neurosci., Boston Univ., Boston, MA

Abstract: Acetylcholine plays an important role in memory. Specifically, administration of the cholinergic antagonist scopolamine has been shown to disrupt the ability to encode new memories (Ghoneim and Mewaldt, 1975; Petersen, 1977). Prominent theta and gamma oscillations can be found in the medial temporal lobe (Bragin et al., 1995; Buzsáki, 2002, 2006; Colgin et al., 2009; Belluscio et al., 2012). Theta modulates the power of gamma oscillations, and this theta-gamma coupling has been proposed to play a role in the routing of hippocampal inputs (Colgin et al., 2009; Schomburg et al., 2014). Furthermore, experiments in our laboratory have shown altered theta-gamma coupling in entorhinal cortex following scopolamine (Newman et al., 2013). Modeling work in our laboratory has suggested how differential modulation of inputs, and thus encoding and retrieval, by theta phase and acetylcholine could be useful for stable memory formation (Hasselmo and Wyble, 1997; Hasselmo et al., 2002). Here, we leverage the temporal and anatomical specificity of optogenetics to explore this hypothesis. Using archaerhodopsin expressed in ChAT positive neurons, we can silence medial septal cholinergic neurons, reducing cholinergic tone selectively in the hippocampal circuit. We use the hippocampally-dependent delayed non-match to position task to investigate the role of acetylcholine in hippocampal memory. This task allows us to repeatably separate encoding and retrieval, and thus we can examine the effect of cholinergic silencing during the different phases of the trial, testing whether septal acetylcholine is required for encoding on the seconds to minutes timescale. Additionally, we have performed recordings using silicon probes with sites spanning layers of hippocampal region CA1 during running. Using current source density and phase-amplitude coupling, we have examined the effects of cholinergic silencing on anatomically sourced hippocampal inputs. Together, these results will provide insights into the role that acetylcholine plays in shaping hippocampal physiology, and the relationship between this physiology and memory processing.

Disclosures: **J.R. Climer:** None. **M.E. Hasselmo:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

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NIMH 5R00MH085944

PEW FOUNDATION

ALFRED SLOAN FOUNDATION

Title: Task-relevant dynamics of neural network activity in mouse prefrontal cortex

Authors: *H.-A. TSENG, X. HAN;
Boston Univ., Boston, MA

Abstract: Decision making process involves prefrontal area to integrate the sensory information, and to convert it to proper decisions based on learnt rules. While primate prefrontal cortex (PFC) has been well characterized for its crucial role in decision making process, mouse PFC is much less understood. As an attractive animal model with increasingly available genetic and molecular tools, mice provide a unique system to link understandings across molecular, genetic, neural network and behavioral levels. To understand how mouse PFC neurons process sensory information during decision making, we characterized PFC activities in freely moving mice performing an auditory discrimination task. In this task, mice were trained to initiate the presentation of one of three auditory cues, and to obtain reward at specific locations according to the cue features. We recorded single neuron activities and local field potentials (LFPs) using tetrodes bundles positioned at four sublocations within PFC, while mice are performing the task. We discovered that PFC neurons exhibit task relevant activities, with distinct neuron populations increasing activities at discrete phases of the behavioral task. Further examination shows that the spike activities could be modulated by the information relevant to the stage of the task where they have highest spike activities. Beside the spike activity, the beta oscillation of LFP is also modulated by the task progress and exhibits two peaks. One right before mice initiated the task, and the other always towards the end of the task. While the timing of the first peak is consistent across the trials, the timing of the second peak is positively correlated with the trial duration. Overall, our results showed that both spike and LFP are modulated by the task progress, and the spike activity could carry the curial information relevant to the task.

Disclosures: H. Tseng: None. **X. Han:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

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PEW FOUNDATION

ALFRED SLOAN FOUNDATION

Title: Cholinergic modulation of cortico-cortical interaction

Authors: N. JAMES[1], H. GRITTON[1], N. KOPELL[2], *X. HAN[1];
[1]Biomed. Engin., [2]Mathematics, Boston Univ., Boston, MA

Abstract: Cortical projecting cholinergic neurons modulate neural circuit interactions over a wide range of spatiotemporal scales, from transient influence on information processing within local brain regions to long term impacts on synaptic plasticity and general arousal across multiple brain areas. Over the years, *in vitro* slice physiology experiments have established a comprehensive understanding of the biophysical mechanisms underlying acetylcholine action on various cell types and local circuit interactions. In addition, *in vivo* pharmacological experiments, via systemic or intracranial infusion of cholinergic agonists or antagonists, have established a direct link between cholinergic activation and attentional processing, sensory detection and plasticity. However, detailed neural circuit mechanisms underlying acetylcholine actions across large neural networks during information processing is less well understood. To examine the functional role of acetylcholine on coordinating cortical-cortical interactions, we performed simultaneous recordings in the auditory cortex and the prefrontal cortex in awake head fixed mice, when mice were presented with auditory stimuli. We discovered that auditory stimuli could effectively elicit responses in the prefrontal cortex during passive listening state, and the responses seen in prefrontal cortex are modulated by muscarinic receptor activation within the auditory cortex. Together our results suggest that acetylcholine can gate information flow from the auditory cortex to the prefrontal cortex selectively via muscarinic receptor mechanisms.

Disclosures: **N. James:** None. **H. Gritton:** None. **N. Kopell:** None. **X. Han:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

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NIMH 5R00MH085944

PEW FOUNDATION

ALFRED SLOAN FOUNDATION

Title: Alpha coherence between frontal and auditory cortex during extinction learning in mice

Authors: *N. JAMES, H. GRITTON, Z. YAO, N. KOPELL, X. HAN;
Boston Univ., Boston, MA

Abstract: Cortical synchrony--a hallmark of executive functioning--is theorized to contribute to communication between networks during cognition. In particular, selective attention and cognitive flexibility--the ability to update task rules when conditions change--are two notable deficits often characterized in individuals with impaired executive function. To understand the functional role of neural synchrony in selective attention and cognitive flexibility, we recorded from auditory (AC) and frontal cortex (FC) while mice performed an extinction learning task. Prior to recording, animals were trained to lick for a small water reward that had been paired with two distinct tones separated by 1.5 octaves. Once the associations were well learned, recordings were made from animals when the reward was withdrawn from one of the two tones while the other was maintained. We observed that frontal neurons show enhanced sensitive to the extinguished tone during extinction learning. In addition, we found that neural synchrony between AC and FC is enhanced broadly during cue processing but that alpha (8-13 Hz) power and coherence is selectively enhanced only for the extinguished tone, and only as mice learned to suppress licking. Taken together, these results suggest that increased coherence between sensory and association regions is an inherent property of auditory discrimination and that alpha oscillations may underlie the processes necessary for cognitive flexibility.

Disclosures: **N. James:** None. **H. Gritton:** None. **Z. Yao:** None. **N. Kopell:** None. **X. Han:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

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Title: Representations of novel and familiar object-place associations differ during slow and fast gamma rhythms in the hippocampus of freely behaving rats

Authors: *C. ZHENG[1], K. W. BIERI[2], L. L. COLGIN[3];
[1]Univ. of Texas At Austin, Austin, TX; [2]Inst. for Neurosci., [3]Ctr. for Learning and Memory, The Univ. of Texas at Austin, AUSTIN, TX

Abstract: Hippocampal gamma rhythms are thought to play a role in memory operations by coordinating the activity of neurons that code related information. Increasing evidence suggests that gamma rhythms split into distinct slow (~25-55 Hz) and fast (~60-100 Hz) gamma subtypes, yet the significance of these different subtypes with regard to memory remains unclear. In this study, we investigated how slow and fast gamma rhythms relate to memory encoding of novel object-place associations and memory retrieval of familiar object-place associations. Place cell spikes and local field potentials were recorded in hippocampal subfields CA1 and CA3 for 6 rats and CA1 for 1 additional rat. Recordings were collected during three different novelty conditions: a novel object placed in a familiar location, a familiar object placed in a new location, and a novel object placed in a new location. The strongest and most consistent behavioral and physiological effects were observed when a novel object was placed in a new location; results from this condition are described below. Fast gamma power in CA1 and CA3, and CA3-CA1 fast gamma phase synchrony, significantly increased during exploration of novel object-place associations compared to familiar object-place associations. On the other hand, CA3-CA1 slow gamma phase synchrony was significantly higher during exploration of familiar object-place associations. Also, hippocampal place cell spikes were more strongly phase-locked to fast gamma during exploration of novel object-place associations than during exploration of familiar object-place associations. Such effects were not observed for slow gamma phase-locking

of place cell spikes. Moreover, CA1 place cells with fields close to novel objects in new locations exhibited significantly higher firing rates during fast gamma epochs than during slow gamma epochs. These results suggest that novelty signals induce fast gamma coordination of hippocampal subfields and corresponding enhancement of place cell firing during encoding of novel object-place associations.

Disclosures: **C. Zheng:** None. **K.W. Bieri:** None. **L.L. Colgin:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

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Esther A. and Joseph Klingenstein Fund

NSF Graduate Research Fellowship Program (to B.J.G.)

Title: Hippocampal place cells exhibit distinct spatial coding modes in mice

Authors: *B. J. GEREKE[1], D. T. JONES[2], L. L. COLGIN[2];
[1]Inst. for Neurosci., [2]Ctr. for Learning and Memory, The Univ. of Texas at Austin, Austin, TX

Abstract: Previous work in rats has demonstrated that 'prospective' and 'retrospective' coding modes exist in grid cells (de Almeida et al. 2012) and place cells (Muller and Kubie 1989, Battaglia et al. 2004, Bieri et al. 2014). In this context, prospective and retrospective modes are defined as times when the majority of a place cell's spikes occur prior to (prospective), or shortly after (retrospective), the animal passes through its place field. In CA1 place cells, retrospective modes have been proposed to support the encoding of recent information, driven by persistent firing in medial entorhinal cortex (MEC). On the other hand, prospective modes are thought to reflect a CA3-dependent memory retrieval of representations of upcoming locations. Consistent with this idea, Bieri et al. (2014) showed that CA1 slow gamma rhythms, thought to reflect inputs from CA3, are more prominent during prospective coding, whereas CA1 fast gamma rhythms, thought to reflect inputs from MEC, are more prominent during retrospective coding. These results are intriguing, but a causal dependence of coding modes on anatomically segregated inputs has not yet been demonstrated. Mice offer the genetic tractability to investigate this question, and thus it is important to determine whether such coding modes are also found in

mice. Here, we demonstrate that CA1 place cells indeed display distinct prospective and retrospective coding modes in mice. As in rats, these modes occurred more frequently than predicted from a distribution of randomly shuffled displacements of place cell spikes. Also, as in rats, pairs of cells were more likely to participate in the same coding mode if they were active at approximately the same time, suggesting that coding modes are coordinated at the population level. Moreover, an increased probability of retrospective events was observed during the first passes through a field in a recording session, consistent with the experience-dependent, backward expansion of place fields shown in rats (Mehta et al. 1997). With these results in place, an investigation of the causal dependence of coding modes on anatomically segregated inputs is now possible.

Disclosures: **B.J. Gereke:** None. **D.T. Jones:** None. **L.L. Colgin:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 85.10/Y2

Topic: F.02. Animal Cognition and Behavior

Support: VICI grant (453-09-002)

NWO(056-14-011)

Title: CA3 slow gamma power is driving CA1 theta phase in rat hippocampus

Authors: *H. JIANG[1], A. BAHRAMISHARIF[2], M. VAN GERVEN[1], K. W. BIERI[3], L. L. COLGIN[3], O. JENSEN[1];
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Abstract: It is well established that theta and gamma oscillations interact in the rat hippocampus. One example is the coupling between theta phase and gamma power in the CA1. This theta-gamma cross-frequency coupling (CFC) might facilitate the transient coordination of local networks on short time scales and integrate the processing between more distant networks. However, it is unclear whether the phase of the theta oscillations drives the gamma activity or whether the gamma activity drives the phase of the theta oscillations. We here apply spectral Granger causality and a novel measure termed cross-frequency directionality (CFD) to

investigate the directional interaction between the hippocampal subregions CA1 and CA3. This CFD measure is based on the phase-slope index (PSI) between the phase of slower oscillations and the power envelope of faster oscillations. The data were recorded from freely exploring rats. Granger analysis revealed that CA3 drives CA1 in the theta and gamma band. Further, the gamma power in CA3 was strongly coupled to the theta phase in CA1. Importantly, the CFD measure demonstrated that CA3 gamma power was driving CA1 theta phase. We conclude that the information flow in rat hippocampus is coordinated by an interaction between theta phase and gamma power. The finding that CA3 gamma power drives CA1 theta phase challenges theories suggesting that hippocampal theta activity coordinates neuronal processing reflected in the gamma band. Rather, it seems as if 'bursts' of gamma band activity in CA3 can phase adjust the recorded theta oscillations in CA1.

Disclosures: H. Jiang: None. **A. Bahramisharif:** None. **M. van Gerven:** None. **K.W. Bieri:** None. **L.L. Colgin:** None. **O. Jensen:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 85.11/Y3

Topic: F.02. Animal Cognition and Behavior

Title: Chemogenetic activation of hippocampal area CA2 neurons increases gamma oscillations in hippocampus and prefrontal cortex

Authors: *G. M. ALEXANDER[1], L. Y. BROWN[2], S. FARRIS[1], C. B. PANTAZIS[3], D. J. LUSTBERG[1], B. GLOSS[1], N. W. PLUMMER[1], P. JENSEN[1], S. M. DUDEK[1];
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Abstract: Gamma oscillations (30-120Hz) occur widely throughout the hippocampal formation where they are believed to play a critical role in the encoding and retrieval of declarative memories by coordinating the activation of local neuronal populations into transient cell assemblies. Gamma oscillations have warranted considerable attention in schizophrenia because individuals diagnosed with the disease display abnormalities in this frequency band during a variety of perceptual and cognitive tasks. Although relatively understudied, the CA2 field of the hippocampus is becoming increasingly recognized as a socio-cognitive hub for processing memories containing socially relevant information. Because impairments in social cognition are

a common symptom associated with schizophrenia, and because CA2 is unique within the hippocampus in its susceptibility to loss of interneurons in schizophrenia, abnormal gamma activity in CA2 emerges as a candidate mechanism for linking the known cellular and network pathologies with the socio-cognitive impairments observed in individuals with schizophrenia. To address the relationship between CA2 neuronal activity and gamma oscillations in hippocampus and prefrontal cortex, we infused adeno-associated viruses coding for a cre-dependent excitatory DREADD (Designer Receptors Exclusively Activated by Designer Drugs) into hippocampi of mice that express cre recombinase selectively in CA2 pyramidal cells under the control of the *Amigo2* promoter. We then implanted electrode arrays to monitor activity of hippocampal and prefrontal cortical neurons before and following administration of the DREADD ligand, Clozapine-N-oxide (CNO), while animals freely behaved in an open field. We found that CNO increased firing of CA2 pyramidal neurons and dose-dependently increased power of local field potentials in the gamma frequency range in both and hippocampus and prefrontal cortex. At the level of the healthy brain, these findings demonstrate that activation of CA2 neurons is sufficient to induce gamma oscillations not only within the hippocampus, but also in the PFC, suggesting that the CA2 circuitry may play into distributed neural networks beyond its primary target, CA1. In the diseased brain, these findings support the idea that the loss of interneurons from CA2 may underlie, at least in part, the abnormal gamma oscillations observed in schizophrenic patients and provide a mechanistic link to the socio-cognitive impairments observed in patients with schizophrenia.

Disclosures: **G.M. Alexander:** None. **L.Y. Brown:** None. **S. Farris:** None. **C.B. Pantazis:** None. **D.J. Lustberg:** None. **B. Gloss:** None. **N.W. Plummer:** None. **P. Jensen:** None. **S.M. Dudek:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

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Program#/Poster#: 85.12/Y4

Topic: F.02. Animal Cognition and Behavior

Support: Lily's Fund for Epilepsy Research Fellowship 2015

Title: Pattern separation of spiketrains by individual granule cells of the dentate gyrus

Authors: *A. MADAR[1], L. A. EWELL[2], M. V. JONES[1];
[1]Neurosci., Univ. of Wisconsin-Madison, Madison, WI; [2]Neurobio., UCSD, San Diego, CA

Abstract: Pattern separation is the process thought to minimize the overlap between patterns of neuronal activity representing similar episodic memories. Theoretical work has suggested that the dentate gyrus (DG) performs this role (O'Reilly; Myers; Aimone) but experimental evidence is incomplete (Leutgeb; Neunuebel; Deng; Ramirez). One of the main limitations has been the difficulty to simultaneously measure DG inputs and outputs *in vivo*, in order to compare the similarity of patterns before and after DG processing. To rigorously assess pattern separation, we have thus developed an electrophysiological assay in the mouse brain slice, which constitutes an accessible platform to stimulate DG afferents and record its output at the same time. Although most studies have considered pattern separation as populations of neurons separating spatial patterns, it is possible that individual neurons of the DG can separate temporal patterns. To test this hypothesis, we generated 12 sets of Poisson impulse trains (~10 Hz), each set consisting of 5 trains with a fixed correlation between each other (0.25 to 1, for 5ms bins). These sets of input patterns were then delivered to the DG, in horizontal slices (400 μm), by focally stimulating the lateral perforant path, while performing whole-cell current-clamp recordings from DG granule cells (GC) (100 stimulus sets for 26 GC). Using Pearson's correlation coefficients as a similarity measure between spiketrains, we found that GC output spiketrains are significantly less correlated with each other than are their driving inputs. Importantly, the decorrelation is higher for very similar inputs than for already dissimilar inputs, consistent with the hypothesized role of pattern separation in memory. Similar results were found using the SPIKE-metric (Kreuz), a bin-less similarity measure based on spike timing. We attempted to rule out whether this form of pattern separation could merely be due to noisy neuronal processing. Shuffling of spikes in original output spiketrains, as well as simulation of output spiketrains based on the mean spike reliability and jitter measured in the original dataset, resulted in outputs significantly more separated but less reliable than the real outputs. This suggests that temporal pattern separation in single GC cannot be explained by trivial random processes, but rather likely involves dedicated biophysical mechanisms. References: Aimone et al. Neuron 61:187. Deng et al. Elife 2:e00312. Kreuz et al. J Neurophysiol 109:1457. Leutgeb et al. Science 315:961. Myers & Scharfman Hippocampus 19:321. Neunuebel & Knierim Neuron, 81:416. O'Reilly & McClelland Hippocampus 4:661. Ramirez et al. Science, 341:387.

Disclosures: **A. Madar:** None. **L.A. Ewell:** None. **M.V. Jones:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 85.13/Y5

Topic: F.02. Animal Cognition and Behavior

Support: KAKEN Grant 26119519

KAKEN Grant 25280051

KAKEN Grant 21300125

Title: Study of the interval timing with administration of NMDA antagonist in rats

Authors: *S. SAKATA[1], A. UJITA[2], M. HATTORI[3];
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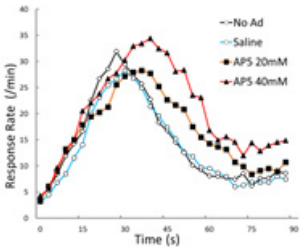
Abstract: Timing behavior is very important to survival and goal reaching in all animals. It is known that animals have some special timing ability of intervals. However, neural mechanisms of time perception are still unknown. The purpose of this study is to investigate the effects of NMDA antagonist on timing behavior. Firstly, using six male rats of Wistar strain, approximately 3 month-old at the beginning of the experiment, we examined psychological expectation of the interval timing in laboratory experimental settings with the peak-interval (PI) procedure. Interval-timing refers to time estimation in the second-to-minutes range. In the PI procedure, rats were trained on a fixed interval schedule to press lever for food after a specified interval (30 seconds in this experiment) as signaled by a certain stimulus. The rats received reinforcement only for desirable response. Though with some individual variations, the distribution of the lever press responses eventually showed an apparent peak in the vicinity of 30 seconds. Secondly, after 30 sessions of trainings, NMDA antagonist was administered directly into the septum region of the brain via microinjection. As a result, the peak time shifted rightward and lever press responses increased. This result of this study suggests that the comparison between the rats administered with NMDA antagonist, NMDA agonist, dopamine agonist and antagonist may clarify neural mechanisms of the interval timing.



Disclosures: **S. Sakata:** None. **A. Ujita:** None. **M. Hattori:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 85.14/Y6

Topic: F.02. Animal Cognition and Behavior

Support: EPSRC

BBSRC

MRC

Title: Theta sequence generation by excitatory-inhibitory interactions in CA1 networks

Authors: *A. CHADWICK, M. VAN ROSSUM, M. NOLAN;
Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: The activity of cells in the rodent hippocampus is strongly modulated by both the location of the animal in an environment and the ongoing theta oscillation. Place cells, but not interneurons, show a strong spatial modulation of their firing rates, while both place cells and some interneurons exhibit phase precession, a phenomenon whereby they spike at a faster frequency than the LFP theta oscillation, causing their spikes to shift to an earlier phase of this rhythm on each successive cycle. Both the mechanisms and functions of phase precession remain largely unclear. We tested whether a minimal model based on the circuit architecture of CA1 and its input from the medial septum accounts for phase precession in place cells and interneurons. In this model place cells and interneurons are reciprocally connected. Interneurons receive pacemaker input from the medial septum, which entrains theta oscillations in the circuit. In simulations of a single place cell and interneuron, we found that phase precession in both cells emerges naturally. When the animal is outside of the place field, the interneuron is fully entrained to the pacemaker theta oscillation and the place cell is rhythmically inhibited, resulting in subthreshold theta oscillations. When the animal enters the place field, the place cell begins to spike, which perturbs the interneuron and causes a transient increase in frequency of the coupled pair of cells. In large scale network simulations, robust phase precession and theta sequences were observed, provided certain constraints on place field mapping and the sparsity of the spatial code were met. In generating phase precession from rate coded place cell inputs, the model we propose suggests that the CA1 circuit flexibly transforms slow rate coded inputs which vary over behavioural timescales into a temporal population code at the timescale of a single theta cycle. The network maintains a short term memory of its inputs, and faithfully preserves their temporal ordering at a much faster timescale suitable for neural processing. Such a network can equally process non-spatial inputs, and can explain why the hippocampus is crucial for the memory of temporal order and transitive relations in general.

Disclosures: **A. Chadwick:** None. **M. van Rossum:** None. **M. Nolan:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

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Topic: F.02. Animal Cognition and Behavior

Support: Marie Curie IEF

ERC Stater Grant

Wallenberg Academy

Ragnar Söderberg Fellowship

Title: Bi-directional optogenetic modulation of prefrontal parvalbumin neuron activity in spatial working memory

Authors: *C. K. YOUNG, M. CARLÉN;
Karolinska Institutet, Stockholm, Sweden

Abstract: Normal brain function requires the coordination of activities from key structures in the brain through oscillation and synchrony. Various implementations of a spatial working memory paradigm in rodents have demonstrated robust coherent activity between the hippocampus and the medial prefrontal cortex at theta (4-12 Hz) frequencies prior to decision making. Parvalbumin (PV+) neurons in the cortex are crucial in coordinating global and local synchrony through feedforward and feedback inhibition. Particularly, putative PV+ interneurons in the prefrontal cortex exhibit spiking entrainment at theta frequencies themselves, as well as entraining local pyramidal neurons during periods of high hippocampo-prefrontal theta coherence through increasing the strength of their inhibitory inputs to pyramidal neurons. In this study, we sought to bi-directionally modulate prefrontal PV+ neuron excitability, hence controlling the level of rhythmic theta entrainment from the hippocampus and ultimately, to enhance or decrease spatial working memory performance. We record caudal hippocampal and medial prefrontal multiunit and local field potential activities from behaving PV-Cre mice injected with either SSFOs (hChR2(C128S/D156A)) or Jaws (Halo57(K200R/W214F)) to increase or decrease PV+ neuron excitability, respectively. The mice were trained to perform a delayed non-matching-to-sample task on an automated figure-of-eight maze. Unilateral optical stimulation was applied during the "choice" phase of the task, namely in the central arm of the maze before committing to right or

left arm visits. We are able to identify a subset of putative PV+ neurons that increased or decreased their spiking activities consistent with the type of opsin activated, and the related changes in spiking activities of putative principle neurons in validation experiments. During the task, optical stimulation to increase or decrease PV+ neuron activities resulted in expected changes in individual neuronal firing rates in the local circuitry (i.e. increase/decrease putative PV+ and pyramidal neuronal firing rates). We are currently investigating changes in theta-entrainment of prefrontal ensembles by optogenetic modulation and effects of optogenetic modulation when applied to other (i.e. delay and consummatory) periods of the task.

Disclosures: C.K. Young: None. **M. Carlén:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH100820

Title: The role of the nucleus reuniens of thalamus in synchronization of oscillations of the prefrontal cortex and hippocampus in urethane anesthetized rats

Authors: *B. KOCSIS, F. PETTERSSON SVENSSON, A. T. ROY;
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Abstract: Recent anatomical studies suggest that while hippocampus (HC) directly projects to prefrontal cortex (PFC) the return projection is indirect, through the nucleus reuniens of the thalamus (nRe). This study provides novel electrophysiological evidence that neuronal transmission from the PFC-to-HC is predominated by a 3-4 Hz signal and is mediated via the nRE, in contrast to the HC-to-PFC drive which is strongest at theta frequency and is mediated by direct projection. Local field potentials (LFP) were simultaneously recorded in the HC, PFC and nRe of 17 rats under urethane anesthesia during short episodes of electrical stimulation (0.1 ms square waves at 100Hz, 10 s duraction) of the nucleus reticularis pontis oralis (RPO). Stimulation intensity was systematically varied, spanning a range from threshold to maximum, identified as intensities where low-frequency theta rhythm appears in the HC and where its frequency no longer increases. The LFP segments were extracted and subjected to FFT to obtain power density spectra. Peak frequencies were identified in the HC and PFC respectively, and the power at these two frequencies was calculated for all three signals. Spectra were normalized

across experiments. Pearson's correlations of peak power at both frequencies were calculated between the PFC and HC. Partial PFC-HC correlations were then calculated in which the influence of the nRE was removed. Stimulation of the RPO generated theta (4-9 Hz) oscillations in the HC and a 3-4 Hz LFP rhythm in the PFC. Both rhythms were often present in the spontaneous LFPs, as well. The frequency of both rhythms showed linear relationships with RPO stimulus intensity. Theta frequency was never an integer multiple of the PFC rhythm. Peak powers also changed in a stimulus-dependent manner. The power of theta rhythm, detectable in all three signals, showed direct correlation with stimulation intensity in the HC and PFC but not in the nRE. Similarly, 3-4 Hz was also detectable in the HC and nRe during RPO stimulation but its power negatively correlated with stimulus intensity in all 3 structures. Thus, the 3-4 Hz signal followed the opposite trend of what was observed the theta signal, suggesting a possible negative coupling between the two rhythms. Furthermore, we found significant correlation between theta power in HC and PFC (0.441) which only slightly decreased after partialization with the theta signal in the nRe (0.357; 19% decrease). In contrast, HC-PFC correlation at the peak of the 3-4 Hz rhythm showed a 97% decrease (from 0.506 to 0.016) after partialization with nRe LFP, suggesting that PFC-HC correlation at 3-4 Hz but not at theta frequency is largely mediated by the nRE.

Disclosures: B. Kocsis: None. **F. Pettersson Svensson:** None. **A.T. Roy:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

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Topic: F.02. Animal Cognition and Behavior

Support: Spanish BFU2011-29286

Spanish BFU2011-29089

Title: A variable oscillator underlies the measurement of time intervals in the medial prefrontal cortex during classical eyeblink conditioning in rabbits

Authors: C. R. CARO-MARTÍN, R. LEAL-CAMPANARIO, R. SÁNCHEZ-CAMPUSANO, J. M. DELGADO-GARCÍA, *A. GRUART;
Pablo de Olavide Univ., Seville, Spain

Abstract: We were interested in determining whether rostro-medial prefrontal cortex (rmPFC) neurons participate in the determination of conditioned stimulus-unconditioned stimulus (CS-US) time intervals during classical eyeblink conditioning. Rabbits were classically conditioned with a delay paradigm consisting of a tone (600 Hz, 90 dB) as CS. The CS started 150, 350, 600, 1100, or 2100 ms before and co-terminated with an air puff (100 ms, 3 kg/cm2) directed to the cornea as US. Eyelid movements were recorded with the magnetic search-coil technique and with the EMG activity of the orbicularis oculi muscle. Unitary recordings of pyramidal neurons were carried out following procedures described elsewhere (Leal-Campanario et al., J. Neurosci., 33: 4378, 2013). For unitary analysis we developed a customized spike sorting algorithm that estimates the number of neuronal spikes distributed across time according to up to twenty-two physiological parameters characterizing each action potential. Reflex, spontaneous, and conditioned eyelid responses presented a dominant oscillatory frequency of about 10 Hz. In its turn, the firing rate of each recorded neuron presented a single peak of activity with a frequency dependent on the CS-US interval (i.e., about 12 Hz for 250 ms, about 6 Hz for 500 ms, andabout 3 Hz for 1000 ms). Interestingly, rmPFC neurons presented their dominant firing peaks at 3 preferred times evenly distributed with respect to CS start, also depending on the CS-US interval (only for intervals of 250, 500, and 1000 ms). No significant neural responses were recorded at very short (50 ms) or large (2000 ms) CS-US intervals. In accordance with the present results, we can conclude that rmPFC neurons do not encode the oscillatory (or kinematic) properties characterizing eyelid responses in behaving rabbits (Gruart et al., J. Neurophysiol., 83: 836, 2000), but are probably involved in the determination of CS-US intervals of an intermediate range (250-1000 ms). We proposed that this variable oscillator underlies the generation of working memories in rabbits, in a way probably different to neural mechanisms underlying working memories in primates.

Disclosures: C.R. Caro-Martín: None. **R. Leal-Campanario:** None. **R. Sánchez-Campusano:** None. **J.M. Delgado-García:** None. **A. Gruart:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 85.18/Y10

Topic: F.02. Animal Cognition and Behavior

Support: MH65561

MH73057

Title: Prelimbic cortex infusion of SSRI fluoxetine reduces the effects of emotional distracters on interval timing

Authors: *A. R. MATTHEWS, M. BUHUSI, C. V. BUHUSI;
Dept. of Psychology, Utah State Univ., Logan, UT

Abstract: Emotional distracters impair cognitive function. Emotional processing is dysregulated in affective disorders such as depression, phobias, schizophrenia, and post-traumatic stress disorder. Among the processes impaired by emotional distracters, and whose dysregulation is documented in affective disorders, is the ability to time in the seconds-to-minutes range, i.e., interval timing (Buhusi and Meck 2005, Nat Rev Neurosci 6:755-65). Presentation of task-irrelevant distracters during a timing task results in a delay in responding suggesting a failure to maintain subjective time in working memory, possibly due to attentional and working memory resources being diverted away from timing, as proposed by the Relative Time-Sharing model (Buhusi and Meck 2009, Philos Trans R Soc B 364:1875-85). Here we investigated the role of the prelimbic cortex in the detrimental effect of anxiety-inducing task-irrelevant distracters on timekeeping, using local infusions of serotonin reuptake inhibitor (SSRI) antidepressant fluoxetine, in procedures previously developed in our lab (Matthews et al. 2012, Frontiers in Integrative Neuroscience 6(111): 1-12). Because in previous research we found that chronic administration of fluoxetine decrease the disruptive effect of emotional events on timekeeping (Christensen et al. 2013, Neuroscience Meeting Planner, Program No. 856), we hypothesized that fluoxetine acts in the prelimbic cortex to reduce the effects of anxiety-inducing distracters on interval timing. Similar to results reported by Matthews et al. 2012 for antidepressant nomifensine, and to previously reported effects of chronic fluoxetine, prelimbic cortex infusions of fluoxetine revealed a dissociation between the effects on interval timing and time sharing (resource allocation), and between neutral and anxiety-inducing distracters. Fluoxetine was effective in a dose-dependent manner only during trials with anxiety-inducing distracters, but not when the distracters were neutral, or in trials without distracters. Results are discussed in relation to the brain circuits involved in the Relative Time-Sharing of resources, and the pharmacological management of affective disorders.

Disclosures: **A.R. Matthews:** None. **M. Buhusi:** None. **C.V. Buhusi:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 85.19/Y11

Topic: F.02. Animal Cognition and Behavior

Title: Latent variable modeling of hippocampal replay

Authors: *E. ACKERMANN, C. KEMERE;
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Abstract: Neuronal firing patterns are often preserved and repeated in a time-compressed manner (so-called replay or neural reactivation), but it remains challenging to (i) identify and (ii) quantitatively assess these events from extracellularly recorded neural data. Much is still unknown about replay, and the ability to reliably detect replay events is of critical importance in order to further analyze the nature of, and functional role of replay in learning and memory. Moreover, a statistical understanding of replay events is still lacking. For example, what percentage of the bursts of neural activity during sharp wave-ripple complexes (SPW-R) in the hippocampus are considered replay events? And what do those sequences represent that are not classified as replay? Most current approaches to detect replay rely on either template matching, or on decoding-based sequence identification, followed by an ad hoc scoring step. Unfortunately these approaches lack a principled framework, making it difficult to extend these approaches to more complicated environments. Furthermore, the lack of a principled framework makes a comparison between different approaches---and the interpretation of results---difficult. Here I show how latent variable models---and hidden Markov models (HMMs) in particular---can be used to identify and quantify putative sequences of reactivation in a principled manner. Using HMMs to detect replay events shows comparable performance to existing approaches (in terms of detection accuracy), but there are a number of key advantages. First, the principled framework makes it straightforward to extend replay detection to arbitrarily complex environments, given enough training data. Second, in contrast to template matching approaches, HMMs allow us to identify replay events that did not explicitly form part of the training data, such as shortcut or exploratory sequences. Third, since the HMM is a generative statistical model, we can use it in a simulation context to answer several interesting questions, such as how many different environments might be needed to lead to the level of variability that we typically observe in neural activity during SPW-Rs. Finally, HMMs allow us to detect replay in regions of the brain where behavioral correlates might not be available---the model parameters including the sequential structure in the data are all learnt directly from the neural data itself. This novel approach to studying replay, along with several extensions such as the use of semi hidden Markov models (where state durations are modeled explicitly), is expected to lead to a deeper understanding of replay and its functional significance in learning and memory.

Disclosures: E. Ackermann: None. **C. Kemere:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

Location: Hall A

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Program#/Poster#: 85.20/Y12

Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 NS071050

Veterans Administration Merit Award I01RX000655

AHA EIA 0940065N

Title: Cortical stroke alters temporal processing of cortico-hippocampal circuits

Authors: *J. W. HE, Y. NISHIJIMA, Y. AKAMATSU, J. LIU;
Dept of Neurosurg., UC San Francisco/SFVAMC, San Francisco, CA

Abstract: Stroke is the major cause of adult disability in the United States. More than half of the stroke survivors experience cognitive impairment in later life. However, the neural mechanism of the post-stroke cognitive impairment is poorly understood. Previously we have shown that experimental stroke via the distal occlusion of the middle cerebral artery (dMCAO) induced persistent electrophysiological changes in the hippocampus and impairment in performing the Barnes Maze test, a hippocampal-dependent cognitive test, suggesting that the hippocampal activity and function is significantly altered after cortical stroke. The aim of our current study was to determine in what aspect the communication between the hippocampus and cortex is affected after stroke. Multichannel extracellular electrophysiology recording was conducted in urethane-anesthetized rats housed in either standard or enriched environment (EE) for one month, beginning one week after either stroke or sham surgery. Our results showed that chronic experimental stroke increased theta phase-shift between the CA1 oriens layer and the nearby parietal cortex (network phase-shift) in the contralateral (intact) hemisphere, and EE attenuated the network phase-shift. At the single-unit level, cross-correlation analyses confirmed that the theta-phase modulation of cortical neurons was altered after stroke, which could be restored by EE. Both regression analysis and simulation data suggested that the network phase-shift altered the spike timing property of cortical units after stroke. Thus, these results suggest that stroke disrupts cortico-hippocampal communication by increasing theta phase-shift and changing the time-varying phase modulation in cortical units, while cognitive enhancement via enrichment attenuates these changes. Our data provide the mechanistic insight in the neural mechanism underlying post-stroke cognitive impairment.

Disclosures: **J.W. He:** None. **Y. Nishijima:** None. **Y. Akamatsu:** None. **J. Liu:** None.

Poster

085. Temporal Processing in Septal, Prefrontal, and Hippocampal Circuits

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Program#/Poster#: 85.21/Y13

Topic: F.02. Animal Cognition and Behavior

Support: Spanish BFU2011-29286

Spanish BFU2011-29089

Spanish BFU2014-56692-R

Title: Synaptic-functional and behavioral states characterizing intrinsic and extrinsic hippocampal circuits during operant conditioning in rats

Authors: *R. SANCHEZ-CAMPUSANO[1], I. FERNÁNDEZ-LAMO[2], A. GRUART[2], J. DELGADO-GARCÍA[2];
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Abstract: It is generally assumed that associative learning is able to evoke more-or-less stable changes in synaptic strengths in selected cortical and subcortical sites. It has also been shown during classical eyeblink conditioning (Gruart et al., Cereb. Cortex, in press, PMID: 24654258, 2014) that these changes depend on the specific and timed activation of multiple hippocampal and other cortical synaptic contacts, evoking a precise map of synaptic-timing states. However, a still-open question is whether synapses included in the intrinsic hippocampal circuit, and those involved in its main inputs (perforant pathway) and outputs (subiculum, prefrontal cortex, and thalamic reuniens nucleus), present similar changing rates in synaptic strength and configure a coherent map of functional states, when the tools of an operant conditioning task are used to modify well-defined behaviors (going to the lever, at the lever, lever pressing, going to the feeder, eating, resting, exploring, grooming) of the animals. Here, we have recorded activity-dependent changes in synaptic strength --namely, the slope of the chronically evoked field postsynaptic potentials (fPSPs), in different intrinsic and extrinsic hippocampal synapses (n = 12) during the acquisition and storage of operant conditioning tasks in alert behaving rats. Rats were trained using a fixed-ratio (1:1) schedule. Furthermore, we have developed an analytical approach of state functions to characterize the learning process and the underlying synaptic changes. We characterize qualitatively and quantitatively two types of evolution patterns for all the synapses: 1) the synaptic-timing changes during each behavior, and (2) the timing-behavioral changes of each synapse. Preliminary results indicate that the acquisition of an operant conditioning task is a multisynaptic process in which the contribution of each identified synapse

is different in strength, and takes place at different moments across the learning process. In addition, the precise functional states of the selected synapses during a given behavior changes in accordance with the learning state, determined by values reached in the learning curve (first two sessions, maximum rate of change, and asymptotic values). In conclusion, the precise and timed activation of multiple synaptic contacts during operant conditioning of behaving rats evokes a well-defined dynamic map of functional states characterizing the acquisition of new motor and cognitive skills and the underlying synaptic plasticity.

Disclosures: R. Sanchez-Campusano: None. **I. Fernández-Lamo:** None. **A. Gruart:** None. **J. Delgado-García:** None.

Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

Support: NIH 1DP2DA035149-01

Title: The role of the dorsomedial striatum in spatial working memory

Authors: *H. AKHLAGHPOUR, J. WISKERKE, J. CHOI, J. AU, I. B. WITTEN;
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Abstract: A comprehensive explanation of how working memory is achieved by the brain will most likely involve the interaction of several cortical and sub-cortical brain regions. The striatum is one of the brain regions implicated in working memory, but its precise role remains unclear. We aim to clarify the role of the striatum by recording from striatal neurons of rats while they perform a delayed non-match to sample task. Extracellular recordings from a total 115 units in the dorsomedial striatum of 9 rats revealed that neurons exhibited sequential peaks in firing rates tiling the duration of the trial. Maximum firing rates were biased towards the beginning of the delay period. Roughly 60% of the recorded neurons transiently encoded the sample stimulus at some point in the trial. For most neurons, information about the sample stimulus reached its maximum around the onset of the delay period. Surprisingly, the times of maximum mutual information with the sample stimulus did not correlate with the peak firing rate times. Our findings suggest that the dorso-medial striatum may have a more crucial role at the start of the delay period, which is consistent with theories that suggest that the striatum is responsible for gating information to working memory networks.

Disclosures: **H. Akhlaghpour:** None. **J. Wiskerke:** None. **J. Choi:** None. **J. Au:** None. **I.B. Witten:** None.

Poster

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DA038392

Title: Ventral striatum represents rewards before the orbitofrontal cortex in the Restaurant Row task

Authors: ***Y. A. BRETON**, B. J. SCHMIDT, A. D. REDISH;
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Abstract: We used the Restaurant Row task to investigate the timing of representations of the flavor-based decision in populations of orbitofrontal cortex (OFC) and ventral striatal (VS) cells. In this task, rats made stay/skip decisions at each of 4 zones in daily, hour-long sessions. Rats ran clockwise around a loop with 4 spokes, at the end of which a feeder provided 2 food pellets of unique flavor (cherry, banana, plain, and chocolate) after a cued delay that lasted 1-30s, randomly offered on each encounter with the zone. Zones were only activated in sequence. As rats entered each zone, a sequence of tones counted down the delay until a reward was delivered or the rat exited, proceeding to the next zone. OFC and VS cells have been proposed to contribute differently to flavor-based decision-making, but the timing of their representations in a foraging task is not known. To determine the timing of representations of flavor offers in our spatially-driven operant task, we recorded from OFC and VS cells using a 24-tetrode assembly. We then used Bayesian decoding methods to infer the probability of representation of the currently-offered flavor, the previously-offered flavor, the next flavor, the flavor presented on the opposite side of the maze, and non-specific activity. The Bayesian algorithm was trained from firing in the first 3s following reward delivery (for each flavor) or firing at all other times (for non-specific activity), in 125ms bins. The test set consisted of firing in the first 5s of zone entry, in 125ms bins. We performed this Bayesian decoding algorithm separately for OFC and reward-responsive VS cells. We then isolated the time course of representations of each goal type (current, next, previous, or opposite) for each choice (stay, skip) in each structure. When

rats decided to wait through the delay until rewarded, representation of the current zone in both OFC and VS increased over the first 5s of zone entry compared to previous, next, and current zones. Two-way ANOVAs showed significant time bin x goal interactions in both structures. Post-hoc tests of decoding to current goal contrasted with the three other goal types showed significant increases for both structures at later time points. To assess the temporal relationship of the increase in current zone representations between the VS and OFC, the decoded probability of representing the current zone was re-aligned to its value when the rat entered the zone for each structure. This analysis revealed that the ventral striatum provided representations of the currently offered flavor before the OFC. The first time point at which VS reliably represented the current goal preceded the OFC.

Disclosures: **Y.A. Breton:** None. **B.J. Schmidt:** None. **A.D. Redish:** None.

Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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JSPS KAKENHI-11J06508

Title: Systemic injection of clonidine, α2-adrenergic receptor agonist, differentiates prospective spatial representation between options in hippocampal neural ensemble activity

Authors: ***S. AMEMIYA**, A. D. REDISH;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Decisiveness is an important aspect of choice behavior. In human studies, clonidine, an inhibitory α2-adrenergic receptor agonist, increases decisiveness by suppressing mental planning of future actions in decision making. In rats, mental planning of future actions has been observed to occur during “vicarious trial-and-error” (VTE) re-orienting behaviors at choice points, as reflected in hippocampal place cell sweeps of representation ahead of the animal during these re-orientation behaviors. Systemic clonidine suppresses VTE. In order to test whether the neural planning events were similarly affected by systemic clonidine, we examined the influence of clonidine on neural activity in the hippocampal CA1 region 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 a subset of trials, clonidine (30 μg/kg) or vehicle was delivered IP 30 min before the run. Hippocampal representations were assessed by applying a one-step Bayesian spatial decoding algorithm to the recorded neural ensemble activity. Consistent with previous studies, clonidine suppressed, but did not eliminate, the occurrence of VTE-like events. Since mental planning is reflected in spatial representation of future paths in hippocampus, we compared spatial decoding probability between the chosen path and the unchosen path at the choice point. Under vehicle, spatial representations showed an increased representation of the chosen path during Non-VTE laps, while spatial representations showed alternating representations of the two options during VTE laps. Clonidine hastened the differentiation of spatial representations of the chosen path from the unchosen path before rats' reaching the final choice point during non-VTE laps, and suppressed the spatial representation of the unchosen path during VTE laps. Our findings further connect hippocampal representations of options with the decision process and suggest a relationship between the behavioral decisiveness seen under clonidine and limited exploration of plans. The fact that animals only explored one option under clonidine (which they then chose) even when performing vicarious trial and error behaviors suggests that the limited hippocampal search process may help drive behavioral outcomes.

Disclosures: **S. Amemiya:** None. **A.D. Redish:** None.

Poster

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MH090188

MH100284

Title: Sequential activity during theta and sharp wave ripples supports flexible decision making

Authors: *A. E. PAPALE[1], M. C. ZIELINSKI[2], L. M. FRANK[4], S. P. JADHAV[3], A. D. REDISH[5];
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Abstract: Two mechanisms have been proposed as hallmarks of planning in the hippocampus. Sharp wave ripple (SWR) sequences and theta sequence sweeps are both serial representations of paths in an environment. Theta sequence sweeps have a (5-12Hz) theta local field potential signature and occur during movement and attentive pausing, while SWR sequences co-occur with sharp wave ripples (150-250Hz) which are most prevalent during immobility and reward consumption. While both types of activity are well suited to guide decision-making, the relationship between them remains poorly understood. We therefore examined the timing and content of these events in the context of CA1 recordings taken from animals performing a T-maze spatial adjusting delay-discounting task Consistent with previous results, flexible decision making at a choice point of the maze was accompanied by a behavior known as vicarious trial and error (VTE) in which the rat tends to pause and look back and forth before proceeding. During VTE, we confirmed previous findings that decoded representations during theta sequence sweeps were more forward of the rat (Johnson & Redish 2007), consistent with a role in searching through future outcomes. In contrast, SWRs occurred during pauses while rats are waiting at food-delivery sites. As the rat prepared to start on the next lap, SWR sequences began to represent future positions ahead of the rat, consistent with previous findings (Singer et. al., 2013; Pfeiffer & Foster 2013). Interestingly, VTE and SWR event frequency was anti-correlated, both within-session and across-session. In both cases, as the rate of SWR events increases, the occurrence of VTE decreases. Within session, these changes occurred during strategy transitions. Across-session, these changes occur in parallel with learning, with VTE and theta sequences more prevalent early and SWR sequences more prevalent later. Finally, in order to test for a causal relationship between SWR occurrence and VTE behavior, we examined data from a working memory alternation task (Jadhav et al 2012). As demonstrated in the companion poster, VTE increased dramatically in rats in which awake SWRs were disrupted. These findings suggest that theta sequence sweeps and SWR sequences serve complementary functions. Disruption of SWR may result in compensatory increases in search through future outcomes when confronted with a choice, and may leave rats temporarily stuck in earlier stages of learning accompanied by VTE.

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Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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Support: DA030672

Title: Functional coupling between ventral striatum and orbitofrontal cortex in rats running a decision task

Authors: *J. J. STOTT[1], A. D. REDISH[2];
[1]Grad. Program in Neurosci., [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Both orbitofrontal cortex (OFC) and ventral striatum (vStr), are engaged during reward-based decision-making tasks. Recent work has shown differences in timing between OFC and vStr activity, but few studies have looked at synchronous activity in these structures, which could provide clues to their respective contributions to the decision-making process. We analyzed local field potential (LFP) signals from simultaneous OFC and vStr recordings in rats performing an economic decision-making task, the Spatial Adjusting-Delay Discounting task. Rats ran along a T-maze track, making binary choices on each lap between a larger, delayed reward, and a smaller, immediate reward. Delay to the larger reward was titrated based on the rat's decisions. On a fraction of laps, rats showed prominent pause and look behavior at the choice point, termed "Vicarious Trial and Error" (VTE). This pause and look behavior was more frequent early in the session, and gradually decreased as rats' transitioned to a fixed 'left-right' alternation strategy later in the session, when the value of both sides was roughly equal. Power spectral density analyses showed a prominent peak at 50 Hz (γ50) for OFC and peaks at both 50 Hz (γ50) and 80 Hz (γ80) for vStr. In both OFC and vStr, γ50 power increased dramatically during reward delivery, while γ80 power dropped precipitously, as has been reported previously for vStr, showing a strong task-dependent difference in these two frequency bands. We found parallel results for the coherence between OFC and vStr LFP signals after reward delivery (increasing for γ50, and decreasing transiently for γ80). We applied two measures of directional connectivity to our data set. Looking at the maximum crosscorrelation between γ-filtered LFPs, we found that LFP gamma oscillations in vStr consistently led those in OFC. Likewise, using Granger Causality (GC) analysis, we found that time-domain GC was greater in the vStr to OFC direction than in the OFC to vStr direction, indicating that vStr more strongly influences OFC, at least on this decision-making task. Spectrally, this influence was strongest at gamma frequencies (40-70 Hz). Interestingly, GC values in the vStr to OFC direction were greater during deliberative VTE events, as compared to non-VTE passes through the choice point, but this was

not the case for GC values measuring OFC influence on vStr. Taken together, these data suggest that vStr strongly influences the OFC in the gamma range, and that vStr more strongly influences OFC during deliberative decision-making modes.

Disclosures: J.J. Stott: None. **A.D. Redish:** None.

Poster

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NSF-1305977

Title: Rats titrate to different adjusted delays on parallel foraging and decision-making (delay-discounting) tasks

Authors: E. C. CARTER[1], D. W. STEPHENS[1], *A. D. REDISH[2];
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Abstract: Behavior depends on consequences that differ through time (intertemporal choice). Several theories have been proposed to explain how organisms balance temporally proximate and more distal consequences. One dominant view is that the value of a future reward is discounted by the delay until its receipt. In contrast, many models from foraging theory predict that organisms have evolved to maximize the overall rate of reward, and thus, opportunity cost drives the preference for immediacy. To compare these hypotheses, we measured the behavior of rats on two tasks with identical reward rates and physical demand: one used to assess delay discounting (DD: choice between a larger-later or a smaller-sooner reward) and one based on foraging (PATCH: choice between investing time at a source of food or moving on). In both tasks, rats ran through a 95cm x 114cm maze with 18cm high LEGO walls forming a square with a central track. Food-delivery sites were placed at the NW and NE corners of the square; one-way doors forced the rats to enter from the top and to leave by the side paths. In DD, the rats ran through the central track to turn left or right. One direction provided 6 pellets after a delay D, while the other direction resulted in 2 pellets after 1s. Choosing the larger-later side increased D by 1s, while choosing the smaller-sooner side decreased D by 1s. In PATCH, one side was

blocked off so the rat ran a single loop. On arriving at the reward, the rat received 2 pellets after 1s. If the rat then stayed for a delay (D-1)s, the rat received 4 pellets. The delay was adjusted as on the DD task with staying equivalent to choosing the larger-later reward. To vary travel between choices, we also ran versions of the tasks for which 3 switchbacks had been added to the return paths. Despite the similarities between the the two tasks, rats titrated D to very different delays (DD: M = 6s, IQR = [3s, 8s]; PATCH: M = 29s, IQR = [26s, 32s]). Adding switchbacks to the return paths increased each titration target (DD: M = 10s, IQR = [6s, 12s]; PATCH: M = 32s, IQR = [29s, 34s]), but there was no evidence of an interaction between task and path length. No models of intertemporal choice simultaneously fit behavior on both tasks, including hyperbolic and exponential delay discounting, long-term and short-term rate maximization, and the recently developed TIMERR and heuristic models. Although each model explained behavior in a single task reasonably well, none explained the pattern of behavior seen in both tasks. This indicates that these tasks may access different decision processes. We suggest that rats (and other animals) may be averse to leaving a cued reward, perhaps because of valuation driven by preference for endowed rewards.

Disclosures: **E.C. Carter:** None. **D.W. Stephens:** None. **A.D. Redish:** None.

Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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MH090188

MH100284

Title: Disrupting awake sharp-wave ripples increases vicarious trial and error behavior

Authors: ***M. C. ZIELINSKI**[1], A. E. PAPALE[3], A. D. REDISH[4], L. M. FRANK[5], S. P. JADHAV[2];
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Abstract: The hippocampal circuit shows sequential activity of place cells representing paths in an environment during two distinct activity patterns, sharp-wave ripples (SWRs, 150-250 Hz) and theta oscillations (5-12 Hz). SWR-associated forward and reverse replay during pauses in behavior has been shown to represent prospective, retrospective and remote trajectory information (Diba & Buzsáki, 2007; Karlsson & Frank, 2009). In comparison, theta sequences are seen during motion and attention, particularly during most mobile behaviors. Theta sequences represent upcoming paths serially at choice points in maze tasks coinciding with vicarious trial and error (VTE) behaviors, during which animals pause and look back and forth (Johnson & Redish, 2007). Current evidence suggests a role for both of these hippocampal mechanisms in planning and decision-making, but their specific roles and the relationship between them are unclear. Here, we show that disruption of awake SWRs during a working memory task leads to increased VTE behavior, demonstrating a causal link between these two phenomena. We examined VTE behavior in rats learning a W-track spatial alternation task. Awake SWRs on the track were disrupted, without altering place cell activity, using real-time detection coupled to electrical stimulation in the ventral hippocampal commissure. We have previously shown that disrupting SWRs impairs memory-guided decision-making on this task (Jadhav et al., 2012). Control animals received similar closed loop feedback stimulation, but with a delay of 150-200ms to leave SWR activity intact. VTE behavior was quantified during outbound trajectories (trials from the center of the W-track outward) that require spatial working memory. We found that SWR disruption animals showed a significantly higher incidence of VTE compared to control animals. Further, SWR disruption animals showed significantly higher VTE in both correct and error trials, and throughout the entire learning period following the first day of acquisition. Loss of SWRs thus led to a compensatory increase in VTE behavior as animals attempted to learn the task. In agreement, we found SWRs and VTE to be anti-correlated in a spatial adjusting delay-discounting task (demonstrated in the companion poster). SWRs and theta sequences during VTE may therefore represent complementary hippocampal mechanisms for planning and deliberation associated with memory-guided decision-making.

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Poster

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Title: Anterior cingulate cortex-hippocampal interactions during goal-driven behaviors

Authors: *J. Y. YU[1], A. LOBACK[2], I. GROSSRUBATCHER[1], D. LIU[1], L. M. FRANK[1]; [1]UCSF, San Francisco, CA; [2]Princeton Univ., Princeton, NJ

Abstract: The brain has the remarkable ability to quickly adjust behavioral choices when faced with unanticipated changes in the world. This ability requires interactions among multiple brain systems, including the hippocampus and the prefrontal cortex (PFC). While neural correlates of planning and decision making have been identified in both the PFC and the hippocampus separately, it is unclear how these brain areas interact to support adaptive behavior. We examined these interactions in rats performing a novel spatial foraging task where changing reward location contingencies require the animals to dynamically adjust spatial trajectory choices. The task was designed to engage the hippocampus and the anterior cingulate cortex (ACC), a part of the PFC critical for adaptive decision making, and inactivation of ACC was sufficient to disrupt trajectory choices without altering motivation. We then recorded from multiple single neurons in both the ACC and the hippocampus of awake, behaving rats to examine the interactions between these structures. We found that network ensemble patterns in the ACC preceding trajectory decisions are dynamic and reflect the operative reward contingency and location. We also found strong modulation in the activity of a large population of ACC neurons coincident with hippocampal sharp-wave ripples (SWR), events during which the hippocampus can reactivate sequences of place cells reflecting possible future trajectories. Furthermore, the pattern of ACC modulation during hippocampal SWRs was also related to the current reward location and contingency. These findings indicate that temporally precise interactions occur between the ACC and the hippocampus at the time of SWRs and that these interactions reflect behaviorally relevant information including location and reward contingency. This mode of cortical-hippocampal interaction could provide a mechanism where information about future trajectory options from the hippocampus is combined with information about reward contingencies to influence adaptive decisions.

Disclosures: J.Y. Yu: None. **A. Loback:** None. **I. Grossrubatcher:** None. **D. Liu:** None. **L.M. Frank:** None.

Poster

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The Kavli Foundation

Title: Spatially periodic firing in grid cells requires local inhibition through parvalbumin interneurons

Authors: *Q. CAO, C. MIAO, E. I. MOSER, M.-B. MOSER;
Kavli Inst. For Systems Neuroscience, CNC, NTNU, Trondheim, Norway

Abstract: Grid cells in the medial entorhinal cortex (MEC) are an essential component of the brain's representation of local space. The maxtrix-like hexagonal firing pattern of grid cells is thought to reflect attractor network mechanisms but the exact implementation remains elusive. A common property of attractor networks is the presence of strong recurrent connections between cells with similar functional properties. In most attractor network models, such connections are excitatory. However, in layer II of the MEC, where most grid cells are located, the most abundant cell type - the stellate cells - is connected exclusively via inhibitory interneurons (Couey et al., 2013). Recent attractor network models have shown that grid patterns can be generated in network where grid cells are connected only by all-or-none inhibitory interneurons, if the network receives steady excitatory input from an external source such as the hippocampus (Couey et al., 2013)(Bonnevie et al., 2013). One prediction of these models is a disruption of the grid pattern if the inhibitory connections between the stellate cells are silenced. We tested this prediction by reducing activity specifically and locally in parvalbumin-expressing interneurons (PV) in the MEC, the main type of inhibitory cell in this area. We used PV-Cre transgenic mice and injected Cre-dependent adenoassociated virus (AAV) expressing the pharmacologically selective designer Gi-protein-coupled muscarinic receptor hM4D (Armbruster et al., 2007). The AAV was injected into MEC. At the same time tetrodes were implanted in the injection area. Three weeks after viral infection, the activity of grid cells was collected while the animal ran freely in a 1 m box both before and after injection of clozapine-N-oxide (CNO, a specific ligand of hM4D). CNO injection caused a clear disruption of the grid pattern. The hexagonal periodicity

was reduced, and firing was dispersed across most of the recording environment. There was no systematic change in firing rate. We are currently testing the effects on border cells and head direction cells, which may possible depend less on the inhibitory connections of the stellate-cell network. The disruption of the grid pattern points to a role for PV-expressing interneurons in the formation of grid patterns in principal cells in the MEC.

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Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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Title: Local deformations in the entorhinal grid cell pattern

Authors: ***M. HAGGLUND**, M.-B. MOSER, E. I. MOSER;
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Abstract: Grid cells are spatially modulated cells that fire in a hexagonal pattern when an animal moves across the environment. Recent studies have demonstrated that, as animals get familiar with an environment, the grid pattern undergoes a rotation and deformation that can fully be accounted for by shear forces along the boundaries of the environment. In large environments, these changes may be local, responding to forces from different boundaries, but the mechanisms underlying these variations are poorly understood. In this study we investigated the interaction between grid cells and the geometry of the environment as animals foraged in differently sized and shaped environments. We applied a sliding window autocorrelation across the recording environment. Grid features were determined locally and related to each other and to the walls and corners of the environment. The autocorrelograms of multiple cells in each position of the map were averaged to gain better resolution and stability. Animals displayed variability in their grid structure throughout the environment. In large recording boxes, the grid was often fragmented so that the grid took on distinct characteristics in different parts of the environment. Typically, the grid had one or more basins where it displayed almost no deviation from

hexagonality. In these locations, the grid tended to align with the wall, with almost no rotational offset, or the axis aligned with the diagonal of the box at 45 degrees. In the rest of the environment, ellipses could be fit to the inner hexagon of the grid pattern. The tilt of these ellipses was not aligned with the axes of the box. Taken together, these findings corroborate the model established earlier that the grid first aligns to the box, but it extends this model by showing how the grid might still retain its default structure in parts of the environment.

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Poster

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The Research Council of Norway

Title: Retrosplenial-parahippocampal projections in the rat are present and adult-like before eye-opening

Authors: ***J. SUGAR**, M. P. WITTER;
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Abstract: Retrosplenial cortex (RSC) is important for a variety of cognitive tasks, particularly within the field of spatial learning and memory. The presence of head-direction cells in rat RSC provides strong support for the notion that RSC plays a role in spatial cognition. Head-direction cells, and other spatially modulated cells are also found in the hippocampal-parahippocampal region (HF-PHR), suggesting a functional relationship. In the adult rat, RSC is connected with pre- and parasubiculum, medial entorhinal cortex, postrhinal cortex and subiculum, areas known to be part of a circuit important for spatial information processing. We have recently shown that sparse connections within the HF-PHR are present at birth, reaching adult-like features before eye-opening and before the first spatially modulated neurons appear at postnatal day (P)15 (Langston et al. 2010 Science 328:1576; O'Reilly et al. 2012 SFN Abstr.702.02). In this study, we aimed to investigate whether RSC connections to PHR develop in parallel with development of HF-PHR connections or in parallel with development of spatially modulated cells in HF-PHR.

To examine development of RSC - PHR connectivity, rats aged P0-P26 were injected with anterograde tracers into RSC. Investigations of anterogradely labeled axons revealed that sparse connections were present at P0. Injections in all parts of RSC, across all postnatal ages, resulted in comparable topology of labeled fibers. During the first week, the connectivity developed gradually and reached adult like densities around P10. We therefore conclude that the topology of RSC - PHR projections is present at birth, while the density of the projections is fully developed during the second postnatal week. Thus, RSC - PHR connections develop in parallel with connections within HF-PHR.

Disclosures: J. Sugar: None. **M.P. Witter:** None.

Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Hall A

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Support: Centre of Excellence scheme of the Research Council of Norway, Grant 145993

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the Kavli Foundation

Title: Topography of place maps along the CA3-to-CA2 axis of the hippocampus

Authors: *L. LU, K. M. IGARASHI, M. P. WITTER, E. I. MOSER, M.-B. MOSER;
Kavli Inst. For Systems Neurosci. and Ctr. For Neural Computation, NTNU, Trondheim, Norway

Abstract: The intrinsic structure of the hippocampus exhibits considerable diversity along the transverse axis, not only between but also within subfields. In CA3, cell morphology and connectivity change gradually from the proximal end (near the dentate gyrus) to the distal end (near CA2), with CA2 appearing in some respects as an extension of CA3 and in others as a distinct subfield with its own gene expression and connectivity patterns. We asked whether the heterogeneity of the CA3-CA2 axis is reflected in how space is mapped onto place cells in these subfields. We observed a transition from cells with small and sharp place fields in proximal CA3 to cells with large and dispersed fields in the most distal CA3 and CA2. The shift was

accompanied by a progressive loss in the ability of place cells to distinguish different configurations of the same spatial environment. The gradual loss of this ability along the CA3-CA2 axis matches several gradients in intrinsic connectivity, such as the increase in recurrent excitatory connections and the parallel decrease in mossy-fiber inputs from granule cells in the dentate gyrus. There was also a reduction along the CA3-CA2 axis in the extent to which place cells formed uncorrelated representations in different places, and in their ability to maintain stable spatial firing over time, but the transition was non-linear, with the sharpest drop within the distal CA3, 200 - 250 μm from the CA2 border. The changes mirror gradients in gene expression and extrinsic connectivity, such as inputs from the supramammillary nucleus, which partly override the cytoarchitectonic CA2-CA3 boundary and extend into distal tip of CA3. The results point to the CA3-CA2 axis as a functionally graded system with powerful pattern separation at the proximal end, near the dentate gyrus, and stronger pattern completion at the distal end, in and near CA2.

Disclosures: L. Lu: None. **K.M. Igarashi:** None. **M.P. Witter:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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Support: Kavli Foundation

Research Council of Norway

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Title: Temporal spike coordination in the prefrontal-thalamo-hippocampal circuit during trajectory decisions

Authors: *H. T. ITO, E. I. MOSER, M.-B. MOSER;
NTNU, TRONDHEIM, Norway

Abstract: Phase synchrony of cortical oscillations is considered as a key mechanism for changing signal flow between regions (Singer, 1999). The connection from area CA1 of the hippocampus to the medial prefrontal cortex (mPFC) is an example of a circuit in which functional connectivity is thought to be modulated by cortical oscillations depending on

behavioral demands. Temporal spike coordination of mPFC neurons relative to theta oscillations in CA1 local field potentials (LFPs) is enhanced when animals run down on the central stem of a T-maze, before they reach the choice point where they have to select one of two routes (Jones and Wilson, 2005; Benchenane et al., 2010). This increase of temporal spike coordination is considered a reflection of enhanced signal flow from CA1 to mPFC. We here report that mPFC and CA1 are theta-coupled also in the reverse direction but with the midline thalamic nucleus reuniens (NR) as a link. In NR neurons, which project to both mPFC and CA1, spikes were found to exhibit enhanced phase-locking to theta oscillations in CA1 LFPs on the central stem, before the decision point, on a T-maze continuous alternation task. Phase-locking was weaker in other regions of the maze. There was also enhanced theta phase coherence between CA1 and NR LFPs on the stem. Our findings point to temporal spike coordination in NR as a potential mechanism for controlling signal flow from mPFC to CA1. This functional connection is essential for CA1 neurons to represent future action plans derived from mPFC (Ito et al., 2015). Thus, by reciprocally coupling mPFC and CA1 cells, theta-frequency spike coordination may enable trajectory decisions during spatial navigation. We are currently exploring the role of the supramammillary nucleus (SUM) as a potential source of behavior-dependent modulation of theta oscillations in the mPFC-NR-CA1 circuit. SUM gives rise to inputs to mPFC, NR and CA2 of the hippocampus, which in turn projects to CA1. We found that many neurons in SUM exhibited rhythmic firing at theta frequency in behaving animals. Some of these neurons increased rhythmic activity on the central stem of the maze, pointing to a key role for SUM in behavior-dependent modulation of theta rhythms in the mPFC-NR-CA1 circuit.

Disclosures: **H.T. Ito:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

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Norwegian Research Council

Title: Commisural connections of the medial entorhinal cortex in rodents

Authors: *K. ZHENG, Ø. W. SIMONSEN, M. P. WITTER;
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Abstract: The rodent medial entorhinal cortex (MEC) contains a variety of spatially modulated neurons, including grid cells, head direction cells, border cells and speed cells. Grid cells are mostly prominent in layer II, as are stellate cells, suggesting that the stellate cell is the candidate grid cell. We have previously reported that stellate cells are locally connected through fast spiking interneurons. The MEC on both sides of the brain are strongly interconnected and these reciprocal connections are topologically organized. *In vivo* recordings have shown that grid cell firing properties are remarkably stable in both hemispheres, pointing to a unified spatial representation in both hemispheres (Stensola et al 2012, Nature 492, 72). Here we studied the commissural connections between the left and right MEC, postulating that these underly this unified spatial representation. We used anterograde and retrograde tracing in mice (n = 13) and corroborated that the main origin of the commissural projection is from neurons in layer III, although a low number of layer II cells contribute as well. The commissural fibers terminate in layers I/II. Using confocal microscopy, we established that commissural fibers target stellate cells and interneurons within layer II. Using an optogenetic approach, we performed multi-cell patch recordings in layer II neurons while optically stimulating commissural inputs which expressed the ChR2 channel. Our results support and extend the anatomical observations and indicate that commissural input might be integrated into the local grid cell network.

Disclosures: K. Zheng: None. **Ø.W. Simonsen:** None. **M.P. Witter:** None.

Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

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Norwegian Research Council Centre for Excellence

Title: Optogenetic characterization of stellate cells in medial entorhinal cortex layer II

Authors: D. C. ROWLAND[1], E. R. SKYTØEN[1], R. NAIR[1], C. G. KENTROS[1], *E. I. MOSER[2], M.-B. MOSER[1];
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Abstract: Layer II of the medial entorhinal cortex (MECII) contains two principal cell types: pyramidal cells and stellate cells. Accumulating evidence suggests that these two cell types have distinct physiological properties and projection patterns. Reelin-expressing stellate cells provide the main excitatory input to the dentate gyrus, CA3 and CA2 region of the hippocampus. The pyramidal cell population forms tight clusters intermingled among stellate cells, earning them the name “island cells”. Pyramidal cells differ from stellate cells in that they express calbindin but not reelin, receive strong cholinergic input, and do not project directly to principal cells in the hippocampus but rather to interneurons in CA1. Together, these observations hint at a fundamental functional difference between the two populations. The currently available data lead to mixed conclusions. Some studies suggest that stellate cells make up a large percentage of grid cell population while others suggest that stellate cells are almost never grid cells. We previously used a tta-based transgenic mouse line to drive expression of arch, an optogentic silencer, in stellate cells (Rowland et al., SfN 2014). Delivering short pulses of light, we found that grid cells and border cells were silenced robustly, indicating that the stellate cell population included grid cells and border cells. However, the relatively low firing rate of the neurons precluded an exact determination of the latency of the response and therefore left the possibility that the cells were secondarily silenced. Here, we introduce new methods for stimulation-based identification of stellate cells. First, we crossed the same transgenic mouse line to a line that expresses reachr, a red-shifted channelrhosopsin variant. Second, we made an AAV virus that expresses Chief, another chanelrhodopsin variant, exclusively in cells that express the tta. The specificity of the latter method can be further improved by injecting the virus directly into the dentate/hilar region and retrogradely infecting sparse populations of reelin-expressing stellate cells. Functional characterization of the cells that express the channelrhodopsin variants is ongoing.

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Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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Center Grant

Title: Entorhinal speed cells project to the hippocampus

Authors: *J. YE[1], S.-J. ZHANG[1,2], E. KROPFF[1,3], M.-B. MOSER[1], E. MOSER[1];
[1]Ctr. for Neural Computation, Kavli Inst. For Systems Neuroscience, NTNU, Trondheim, Norway; [2]Tsinghua-Peking Ctr. for Life Sciences, IDG-McGovern Inst. for Brain Res., Sch. of Life Sciences, Tsinghua Univ., Peking, China; [3]Leloir Institute, IIBBA - CONICET, Buenos Aires, Argentina

Abstract: Hippocampal place cells receive input from multiple functionally specialized spatial cell types in the medial entorhinal cortex (MEC), including grid cells, head direction cells and border cells, with grid cells as the main contributor (Zhang et al., 2013). These cell types are sufficient to map instantaneous position but for activity to be translated from one group of active cells to another, in a way that reflects the animal's movement in the external environment, the cells must have access to information about the current speed and direction of the animal. Recent work has shown that running speed is represented in the firing rate of a ubiqutous population of cells with linear and context-invariant responses to running speed (Kropff et al., SfN 2014). Such ´speed cells´ were identified in both MEC and hippocampus, albeit with slightly different temporal dynamics. In the present study, we asked if hippocampal cells receive speed information from speed cells in the MEC. We used a combined optogenetic-electrophysiological strategy to determine whether hippocampal projection neurons in MEC include speed cells. Retrogradely transportable recombinant adeno-associated virus expressing Flag-tagged channelrhodopsin-2 (ChR2) was injected in the dorsal hippocampus. Virally transduced and optogenetically tagged cells were identified in MEC as cells that fired at fixed minimal latencies of 9-10 ms in response to local flashes of 473-nm blue laser light. Speed cells were identified as cells with a Pearson product-moment correlation between instantaneous firing rate and running speed that exceeded the 99thpercentile of a shuffled distribution of spike times. The population of MEC neurons with short and reliable response times included speed cells, indicating that these cells project directly to the hippocampus. More than 50% of the hippocampus-projecting speed cells had interneuron-like firing properties (narrow waveforms and high firing rates). The population of responsive cells also included neurons with longer response times (up to 30 ms, mostly putative interneurons), suggesting that additional speed cells were stimulated synaptically. The long tail of the latency distribution for speed cells contrasts with the distributions for grid cells, border cells and head direction cells, which were sharp and unimodal, with peaks at 9-10 ms. Taken together, the observations point to entorhinal speed cells as a key component of the MEC input to the place cells in the hippocampus.

Disclosures: J. Ye: None. **S. Zhang:** None. **E. Kropff:** None. **M. Moser:** None. **E. Moser:** None.

Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

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Title: Clonal architecture of the network supporting spatial representation in the medial entorhinal cortex

Authors: *F. DONATO, R. I. JACOBSEN, M.-B. MOSER, E. I. MOSER;
Kavli Inst. For Systems Neurosci., Trondheim, Norway

Abstract: The medial entorhinal cortex (MEC) contains the basic elements of the brain's representation of space, most notably the grid cells, which fire at the vertices of a periodic hexagonal array that covers the entire available environment. Attractor network mechanisms, relying on selective connections among neurons tuned to specific spatial features, have been proposed to account for the emergence of the grid pattern. Since the coordinated maturation of clonally related cells produces preferential connectivity between functionally similar neurons (a feature of the attractor network) in other cortical areas, we took a developmental approach to study MEC microcircuits. By systematic labeling of dividing cells during neurogenesis, or targeted in-utero infection of post-mitotic neuroblasts, we primed cohorts of neurons born in specific time windows for further manipulation, in order to determine their connectivity and function in the adult. We found that stellate cells were anatomically distributed in a dorso-ventral gradient reflecting their neurogenesis, with early born cells located in the dorsal pole of the MEC, and later born cells at progressively more ventral positions. In stark contrast, pyramidal neurons did not show any developmental topography. Despite anatomical overlap between consecutive waves of neurogenesis, clonally related cohorts of stellates cells exhibited stereotyped distributions along the dorso-ventral axis, which were conserved between different animals, thereby recapitulating the spatial organization of grid modules. These data raise the possibility that the temporal organization of stellate cell-neurogenesis might a) set the schedule for the coordinated maturation of clonally related cells into functionally distinct subpopulations; b) determine selective connectivity for the formation of independent microcircuits and hence c) lay the basis for the presence of functionally segregated subnetworks underlying the modular organization of grid cells.

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Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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Title: Speed cells in the medial entorhinal cortex

Authors: *E. KROPFF CAUSA[1,2], J. E. CARMICHAEL[3,2], E. I. MOSER[2], M.-B. MOSER[2]; [1]Leloir Inst. - IIBBA - CONICET, Buenos Aires, Argentina; [2]Kavli Inst. For Systems Neurosci. and Ctr. For Neural Computation, NTNU, Trondheim, Norway; [3]Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Grid cells in the medial entorhinal cortex (MEC) and surrounding parahippocampal areas are unique in their spatial code (Hafting et al, 2005). Unlike other place-modulated neurons, their population firing pattern not only repeats periodically within a given environment, but also seems to apply equally to all explored environments (Fyhn et al, 2007), reflecting the uniformity of space despite the unevenness of contextual details. This property makes grid cells ideal candidates for a path integration-based representation of space (McNaughton et al, 2006). In such a scheme, running speed is integrated across short time windows to obtain the instantaneous displacement of the animal,which in conjunction with head direction input is used to update the representation of the animal´s position. Any path integration mechanism thus requires running speed as a major input.However, while speed has been reported to correlate marginally with entorhinal theta frequency (Jeewajee et al, 2008) and firing rate of grid cells (Sargolini et al, 2006; Wills et al, 2012), the existence and nature of a reliable and locally available speed signal has remained unclear. We found that running speed is represented in the firing rate of a ubiquitous but functionally dedicated population of MEC neurons (~15%) with low levels of overlap with other MEC populations, such as grid, head direction and border cells. Speed cells are characterized by a positive, linear response to running speed, with a great variability in slope and y-intercept. This response is context-invariant, so that running speed can be decoded from speed cell activity even across rooms or in darkness without loss of accuracy. Speed cells and grid cells share a similar prospective bias of around 50 to 80 ms. In both cell types, prospective activity is highly modulated by theta rhythm, suggesting that path integration could take place on a theta cycle basis (Navratilova et al, 2012). In the hippocampus, speed cells were also identified in a smaller proportion (~10%) and with a higher overlap with the place cell population. These cells, however, had a retrospective behavior, while place cells had no temporal bias, a combination that seems to be incompatible with path integration. Taken together, these

observations point to MEC speed cells as a key component of the dynamic representation of self-location.

Disclosures: **E. Kropff Causa:** None. **J.E. Carmichael:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

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The Kavli Foundation

Title: Hippocampal remapping after partial inactivation of the medial entorhinal cortex

Authors: *C. MIAO, Q. CAO, H. T. ITO, H. YAMAHACHI, M. P. WITTER, M.-B. MOSER, E. I. MOSER;
Kavli Inst. For Systems Neuroscience, CNC, NTNU, Trondheim, Norway

Abstract: Hippocampal place cells undergo remapping when the environment is changed (Muller and Kubie, 1987; Colgin et al., 2008). The mechanism of hippocampal remapping remains elusive but spatially modulated cells in the medial entorhinal cortex (MEC) have been identified as a likely contributor. Using pharmacogenetic and optogenetic approaches, we tested the contribution of MEC cells by examining in mice whether partial inactivation in MEC shifts hippocampal activity to a different subset of place cells with different receptive fields. Local infusions of AAV virus were used to express the pharmacologically selective designer Gi-

protein-coupled muscarinic receptor hM4D (Armbruster et al., 2007) or the light-responsive microbial proton pump archaerhodopsin (ArchT) (Han et al., 2011) in the MEC, and place cells were later recorded in the CA3 of the hippocampus after application of the inert ligand clozapine-N-oxide (CNO; in hM4D-expressing mice) or light at an appropriate wavelength (in ArchT-expressing mice). CNO and light both caused partial inactivation in MEC. hM4D and ArchT were expressed across widespread regions of MEC, covering most of its dorsolateral and mediolateral extent, but the inactivation caused only minimal changes in the spatial firing pattern of place cells in CA3. However, while spatial firing was maintained, the distribution of firing locations was altered following even quite restricted silencing in the dorsal parts of MEC. Partial MEC inactivation caused substantial changes in hippocampal spatial representation at the neural ensemble level, reminiscent of the global remapping that occurs in place cells when animals move from one environment to another. The results point to MEC input as an element of the mechanism for remapping in place cells.

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Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

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Topic: F.02. Animal Cognition and Behavior

Title: Grid synchronization in merged space

Authors: *T. WERNLE, M. MØRREAUNET, E. I. MOSER, M.-B. MOSER;
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Abstract: Natural environments are compartmentalized and likely represented by multiple local maps stitched together at salient landmarks (Derdikman et al., 2009). The mechanisms for generating coherence between discrete grid maps have not yet been established. To search for these mechanisms, we trained rats in two rectangular compartments A and B (each 1x2m) separated by a wall. Once two distinct maps were established we removed the wall, allowing the rat to explore the merged open square box (2x2m). Exploration in the large environment was not accompanied by de novo grid formation. Instead, the grid matrices were similar to either one or both of the rectangular maps A and B. In order to reveal the contribution of the initial representations to the global grid pattern in the entire environment we performed several analyses. First, we assessed local similarity between maps using a sliding-box correlation

approach. This approach was tested on a large artificial dataset of rate maps in order to determine optimal bin size for different grid scales and expected range of correlation values for all possible solutions. Subsequent analysis of real data showed that the open field grid pattern was rarely a simple extension of one map but rather a complex fusion of the A and B maps. Fusions could be achieved globally by phase shifting the A and B maps relative to each other or locally by merging the maps at the transition in the center of the box. The type of response was specific to grid modules and global and local fusions could occur simultaneously. Fusion responses required successive visits to the merged environment. In ongoing analyses we are testing the change in local periodicity across the entire environment and we are comparing real field locations to expected field locations of extended A and B maps. The data suggest so far that distinct grid maps for large environments get synchronized both globally and locally, that the effects depend on experience, and that the type of response depends on grid scale.

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Poster

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Title: Body and world-centered coordinate transformations in the parietal-hippocampal network

Authors: *A. A. WILBER[1,2], I. SKELIN[1,2], B. L. MCNAUGHTON[1,2];
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Abstract: A preponderance of evidence suggests that the internal map of the environment is represented in world-centered (allocentric) coordinates; however, movements and perception of the environment are in body-centered (egocentric) coordinates. Therefore, to navigate in space, it is essential to translate between egocentric and allocentric coordinates. Cell activity in the parietal cortex and hippocampus suggests a parietal to hippocampal circuit for transforming landmark representations from egocentric to allocentric coordinates (Wilber et al., 2014),

consistent with theoretical modeling work (McNaughton et al., 1995). A more recent computational modeling predicts two conjunctive representations that are utilized for bi-directional coordinate transformation (Byrne et al., 2007). We described one of these conjunctive representations in parietal cortex for translating from egocentric to allocentric coordinates previously (body centered x head direction). In addition, the computational model predicts a second conjunctive cell type for allocentric to egocentric translation: allocentric landmark direction x head direction. Therefore, we set out to look for evidence that the parietal-hippocampal network also participates in allocentric to egocentric transformation. To do this we recorded single cells in the parietal cortex and hippocampus while rats performed a random spatial sequence task with 32 reward locations arranged around the perimeter of a platform. Each reward location was marked as active with a blinking light, which acted as a landmark. Using lights as "landmarks" allowed for good coverage of landmark directions and headings. As we and Deshmukh and Knierim (2013) previously reported, cells in the hippocampus encode the allocentric direction x distance of landmarks. We also found cells in the parietal cortex that exhibit the properties predicted for allocentric to egocentric transformation, cells that signaled a specific combination of allocentric landmark location and head direction. From such cells the body-centered location of a landmark could be "read out." Interestingly, we previously reported parietal cortex cells which specifically signal body centered landmark direction. Thus, we have extended our previous findings describing a parietal cortex-hippocampal circuit for transforming landmarks from egocentric to allocentric coordinates. We now show that this circuit is part of a larger network that is also capable of translating from world- to body-centered coordinates.

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Poster

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NERF to BLM

Title: A methodological pipeline for serial-section imaging and tissue realignment for whole-brain functional connectomics

Authors: *B. J. CLARK[1,2], L. MESINA[2], A. A. WILBER[3,2], A. DEMECHA[2], C. E. L. STARK[3], B. L. MCNAUGHTON[3,2];
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Abstract: Understanding the neurobiological basis of cognition and behavior, and disruptions to these processes following brain injury and disease, requires a large scale assessment of information exchange amongst populations of neurons, as well as knowledge of their patterns of connectivity. We present an approach that allows for a large-scale investigation of brain connectivity. Animals received unilateral microinjections of retrograde tracers (FluoroGold or cholera toxin-B Alexa Fluor 594) in dorsal cortex and were sacrificed 2 weeks after microinfusion (for details see Wilber et al 2015 Frontiers in Neural Circuits). Serial-section images of whole-mounted brains were acquired during vibratome sectioning in the coronal plane. Tissue sections were then collected and immunohistochemistry was conducted to label neuronal cell bodies and immediate early gene product. Subsequent wide-field fluorescent scans of whole tissue sections were acquired using NanoZoomer whole-slide scanning microscopy (NanoZoomer Digital Pathology RS, Hamamatsu Photonics), which is capable of automatically capturing wide-field multispectral fluorescent images over entire brain sections at high resolution. A custom software platform was developed in Matlab which utilized the open-source software platforms ANTs (http://picsl.upenn.edu/software/ants/), which is a diffeomorphic, vector-field based registration software, the open-source image processing toolkit, Fiji (http://fiji.sc/Fiji), and the neuronal segmentation software, FARSIGHT (www.farsight-toolkit.org). This platform will accommodate an automated analysis pipeline in which neuronal boundaries are automatically identified through segmentation and the x- y- position in each image is co-registered with corresponding serial images acquired during vibratome sectioning. This tool will allow for the representation of whole brain data in 3-D, and the development of methods for rapid segmentation and detection of tracer. We will address the informatics problem of dealing with data from a large number of serial sections and extend this method for detection of multiple immediate early gene products (i.e., measures of functional activation of discrete neuronal populations at single-neuron resolution). This work will lend itself uniquely to analyze both structure and function across entire brains, and will form the basis of a large-scale understanding of the effectiveness of treatments for brain injury and disease.

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Poster

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Title: Low and high frequency electrical perforant pathway stimulation as conditioned stimuli for active avoidance learning: A combined fMRI and electrophysiological study

Authors: *S. RIEMANN, F. ANGENSTEIN;
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Abstract: Direct electrical stimulation of the right perforant pathway (PP) was used as conditioned stimulus to learn an active avoidance task in a shuttle box. To monitor learning related changes in dentate gyrus and whole brain activity, electrophysiological recordings and functional magnetic resonance imaging (fMRI) were combined. In naïve, medetomidine sedated rats the right PP was electrically stimulated and the resultant activity in the granular cell layer of the dentate gyrus was recorded by measuring extracellular field potentials. Simultaneously changes in whole brain activity were determined by measuring blood oxygen level dependent (BOLD) signal intensities. Two different stimulation protocols (20 consecutive trains, train duration 8 s with an inter-train interval of 52 s) were used for electric stimulation of the PP. One train consisted of 8 bursts of 20 pulses each at 100 Hz repetition rate, i.e., 200 ms burst duration, inter-burst interval 800 ms, thus 1 burst per second (100Hz protocol) or continuous 5 pulses per second (inter-pulse interval 200 ms) for 8s (5Hz protocol). The same protocols were then used as conditioned stimulus for an active avoidance task during the following three days (1 hour of 60 trials per day). PP activation preceded a foot shock, thus by jumping over a hurdle after PP stimulation the rat could avoid a foot shock. This means that the initially meaningless PP stimulation became behaviorally relevant during shuttle box training. One day after the last training session, i.e., day 4, the initial combined electrophysiological fMRI measurement was repeated. Rats performed better in shuttle box learning with the 100Hz than with the continuous 5Hz protocol but both groups learned the task. In naïve rats the 100Hz protocol caused a significant BOLD signal in the hippocampus and in the enthorinal cortex whereas the 5Hz protocol led to a BOLD-signal more restricted to the site of stimulation. In trained rats significant BOLD responses were observed in target regions of the hippocampus, such as the septum, nucleus accumbens, and anterior cingulate cortex/medial prefrontal cortex regions during 100Hz but not during 5Hz stimulation protocol. Without additional training the BOLD response pattern remained similar at day 13 when the measurement was repeated. Both groups remembered the task when tested one day later (day 14).

Disclosures: **S. Riemann:** None. **F. Angenstein:** None.

Poster

086. Cortical and Hippocampal Circuits: Spatial Navigation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 86.24/Y37

Topic: F.02. Animal Cognition and Behavior

Title: From default to adjusted. How BOLD fMRI responses relate to neuronal activity in the rat hippocampus during repetitive stimulations

Authors: *F. ANGENSTEIN, C. HELBING, S. RIEMANN;
Deutsches Zentrum Für Neurodegenerative Erkrankungen (DZNE), Magdeburg, Germany

Abstract: During repetitive electrical stimulations of the right perforant pathway concurrent measurements of field potentials in the dentate gyrus and blood oxygen levels dependent (BOLD) signal intensities in the hippocampus were performed to disentangle the role of incoming activity (defined by the applied electrical pulses) and outgoing activity (i.e., spiking activity of the principal neurons measured as population spike) for the formation of a BOLD response. The stimulation pattern were modified that A) the same amount of incoming pulses generate different number of population spike, B) different amount of incoming pulses generate the same number of population spikes and C) increasing number of incoming pulses caused concurrent increases in the number of population spikes. Most of the used stimulation protocols caused variable BOLD responses during repetitive stimulations. Generally, the first stimulation train caused a substantial BOLD response that was followed by an ongoing elevated BOLD signal intensity, which in turn obscured a putative BOLD response to the second train. Thereafter BOLD responses recovered during the following 2 trains and then declined continuously during all succeeding trains, however with a different rate. Based on this general BOLD time series, the response during the first train was considered as response of the naïve dentate gyrus/hippocampus to the stimulus, whereas the responses to the following 3 trains were considered to reflect a reorganization of intrahippocampal circuitry to the incoming stimulations and the responses to the following trains were considered as an adaptation of intrahippocampal circuitry to these repetitive stimulations. Although the stimulation parameters varied considerably, the BOLD response to the first stimulation train was almost identical for 7 out of 10 stimulation protocols, thus in the naïve hippocampus the BOLD response does not relate to electrophysiological parameters of neuronal activity; it rather reflects a default BOLD response to a complex stimulus. However, after reorganization of intrahippocampal circuitry the BOLD

response relates either to the incoming or outgoing activity. Based on these results we present first conservative conclusions about altered neuronal activations that can be drawn from measured BOLD responses in the hippocampus.

Disclosures: F. Angenstein: None. **C. Helbing:** None. **S. Riemann:** None.

Poster

087. Emotion: Brain Imaging

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 87.01/Y38

Topic: F.01. Human Cognition and Behavior

Support: Tamagawa University GCOE Program

JSPS Next Generation World-Leading Researchers Program

Takeda Science Foundation Grant

MEXT Scholarship Program

Title: Understanding emotion in the brain: Comparing categorical and dimensional models of emotion using multivariate pattern analysis

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Abstract: The study of emotion has given rise to two main views about how to model affect: categorical and dimensional. The first view classifies emotions into discreet categories (i.e. anger, surprise, happiness, etc.) based in motivational systems while the other view suggests that emotions are dimensional and can be decomposed into factors such as valence, arousal and control. This study aims to examine the how emotions are represented in the brain using multivariate pattern analysis (MVPA), a variation of functional magnetic resonance imaging (fMRI). Subjects were scanned while they watched emotional videos and rated the emotional experience elicited by the videos both categorically and dimensionally. Behaviorally, subjects were able to rate the emotions they experienced using both categorical and dimensional scales, supporting the idea that both types of emotion can be consciously represented. fMRI analyses using the general linear model revealed parametric effects of emotional dimensions in the ventromedial prefrontal cortex (valence) and intraparietal sulcus (arousal). MVPA analysis found greater than chance level decoding accuracy of both categorical and dimensional emotions,

suggesting that that both types are intrinsically represented in the brain. Meanwhile, results obtained by using a sliding-window MVPA searchlight suggest that differences may exist in the course of how categorical and the various dimensional emotions arise in the brain. Interestingly, average decoding accuracy across subjects for categorical emotions and the valence dimension increased as videos progressed, while accuracy for the control dimension increased until reaching a peak just after the mid-point of videos, followed by a subsequent decrease. For the arousal dimension, accuracy was constantly high. The accuracy increases in categorical emotion and valence may reflect accumulation processes, while the control result suggests the exertion of control during the occurrence of emotional events in videos. Results of the sliding-window searchlight also suggest that regions involved in creating the emotional experience change dynamically throughout the course of emotional development. Together, these results emphasize the fluidity and complexity of emotions, urging us to take a comprehensive view incorporating both models.

Disclosures: **J.C. Foo:** None. **K. Sakai:** None.

Poster

087. Emotion: Brain Imaging

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 87.02/Y39

Topic: F.01. Human Cognition and Behavior

Title: Control of attentional gain modulation by affective valence

Authors: ***X. ZHANG**, Z. SAFIULLAH, S. JAPEE, L. G. UNGERLEIDER;
Lab. of Brain and Cognition, Natl. Inst. of Mental Hlth., Natl. Inst. of Hlth. (NIH), Bethesda, MD

Abstract: The normalization model of attention proposes that attention can affect performance by contrast or response gain changes, depending on the stimulus size and the relative size of the attention field. Here, the attention field was manipulated by affective valence, positive faces versus negative faces, while the stimulus size was fixed in a spatial cueing task. Emotional faces served as a cue to attract spatial attention, followed by a pair of gratings. Subjects performed an orientation discrimination task on one of two gratings; for each of five contrasts (the contrasts of both gratings were identical on any given trial and covaried across trials in random order). A response-cue at stimuli offset indicated the target location, yielding valid cue (the emotional face matched response-cue) and invalid cue (mismatched) conditions. Comparing performance accuracy (*d'*) for valid and invalid trials revealed the spatial cueing effect for each contrast. The

measured psychometric function for each affective valence (positive and negative) and each trial condition (valid and invalid) was fit with the standard Naka-Rushton equation. Two parameters d'_{max} (asymptotic performance at high contrast levels) and c_{50} (the contrast yielding half maximum performance) determined response gain and contrast gain, respectively. We observed a change in the spatial cueing effect consonant with a change in contrast gain for positive faces and a change in response gain for negative faces. Significantly, individual differences in self-reported emotional strength of positive and negative faces correlated with contrast and response gain changes, respectively. An ongoing functional magnetic resonance imaging experiment confirmed that subjects' attention fields were wider for positive faces than that for negative faces. Together, these findings suggest that emotional attention shapes perception by means of the normalization framework.

Disclosures: **X. Zhang:** None. **Z. Safiullah:** None. **S. Japee:** None. **L.G. Ungerleider:** None.

Poster

087. Emotion: Brain Imaging

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Program#/Poster#: 87.03/Y40

Topic: F.01. Human Cognition and Behavior

Support: JSPS Grant 25730091

JSPS Grant 22220003

Title: Valence-general attentional resource for emotional information revealed by activation of the ventral part of the anterior cingulate cortex

Authors: *T. MINAMOTO[1], M. OSAKA[2], N. OSAKA[3];
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Abstract: The ventral part of the anterior cingulate cortex (vACC) is shown to activate under emotional conflict raised by the emotional Stroop task. Taking advantage of the nature of the vACC, the present study examined characteristic of attentional resource for emotional information. Specifically, we tested whether attentional resource is organized in a valence-based manner (i.e., separate attentional resource for negative and positive valence) or valence-general (i.e., common attentional resource regardless of emotional-valence). Combining the emotional working memory (WM) task with the emotional Stroop task, we measured activation of the

vACC with fMRI. Participants were randomly assigned to the negative WM task group or positive WM task group (n = 16 in each group). In the task, participants were instructed to memorize a photograph with emotional content (high intensity or low intensity), which was followed by the emotional Stroop task. In the Stroop task, participants judged a color of a emotional word stimulus whose valence was either negative or positive. If attentional resource for emotional information is arranged in a valence-based manner, activation of the vACC would be smaller when emotional valence of the memory item (especially high emotion) and Stroop stimulus was congruent due to competition for the resource while such pattern would not be observed when valence of the two stimuli was incongruent. On the other hand, if the attentional resource is arranged in the valence-general, activation of the vACC would be smaller when high emotional WM load is given in comparison to the load regardless of the emotional valence. The result was in line with the latter hypothesis; therefore, attentional resource for emotional information may be arranged in valence-general manner.

Disclosures: **T. Minamoto:** None. **M. Osaka:** None. **N. Osaka:** None.

Poster

087. Emotion: Brain Imaging

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 87.04/Y41

Topic: F.01. Human Cognition and Behavior

Title: Neural substrates of romantic love: a positron emission tomography study

Authors: *A. T. SASAKI[1,2], K. MIZUNO[1,2,3], K. TAKAHASHI[1,2], Y. WADA[1,2], M. TANAKA[2], A. ISHII[2], K. TAJIMA[1], N. TSUYUGUCHI[2], K. WATANABE[1], S. ZEKI[4], Y. WATANABE[1,2,3];
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Abstract: When we spend time with a partner in romantic relation, we often feel excitement and healing. Previous functional magnetic resonance imaging study showed the neural activity in the medial insula, anterior cingulate cortex, and striatum, which were known to be involved in the dopaminergic system, related to the romantic love. In the present study, we tested the hypothesis that dopaminergic system was activated when viewing portraits of romantic partner by using positron emission tomography (PET) with [^{11}C]raclopride, a dopamine D_2/D_3 receptor antagonist. We recruited ten normal volunteers who had a romantic partner. During PET scan,

they passively viewed portraits of their romantic partners and, as a control, of friends of same sex for whom they had neutral feelings during the PET scan. The presentation of visual stimulus was started at 15 minutes before administration of [^{11}C]raclopride, and lasted for 30 minutes. The results showed statistically significant activation of dopaminergic system in the medial orbitofrontal cortex (mOFC) and the medial prefrontal cortex (mPFC), when viewing portraits of romantic partner compared with those of friends, the former has been strongly implicated in a variety of rewarding experiences, including that beauty and love. A positive correlation was found in the mOFC between excitement level and dopaminergic activation only for the romantic partner not for friends. Thus, these results suggested that attachment to romantic partner might be represented as dopaminergic activity in the reward system. In the future, it is necessary to investigate whether attachment to romantic partner affects on mental or behavioral performance in such as the mentally exhausted situation.

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Poster

087. Emotion: Brain Imaging

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 87.05/Y42

Topic: F.01. Human Cognition and Behavior

Support: EMDR Research Foundation

Title: Investigating the effect of goal-directed eye movements during extinction on amygdala activity and long-term expression of fear memory

Authors: *L. D. DE VOOGD[1,2], J. W. KANEN[1], K. ROELOFS[1,3], G. FERNÁNDEZ[1,2], E. J. HERMANS[1,2];
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Abstract: Eye movement desensitization and reprocessing (EMDR) is seen as a promising treatment option for fear-related psychiatric diseases such as post-traumatic stress disorder (PTSD). Indeed, experimental studies have shown that performing goal-directed eye movements following recall of autobiographical episodic memories can reduce the vividness and

emotionality of these memories. However, the effect of goal-directed eye movements on retention of fear memory has not been tested directly, and critically, the mechanisms by which eye movements may affect mnemonic processes remain poorly understood. Previous research suggests that performing tasks involving endogenous attention suppresses neural activity in the default mode network (DMN), a large-scale brain network which includes the ventromedial prefrontal cortex (vmPFC) and medial temporal lobe regions such as the amygdala and hippocampus. These regions are also critically implicated in fear and safety learning. We therefore hypothesized that deployment of endogenous attention during goal-directed eye movements suppresses activity in these regions and might thereby affect fear retention. To address this hypothesis, twenty-four healthy volunteers were tested in a Pavlovian fear conditioning paradigm, the most widely used experimental laboratory model of fear and safety learning, which is used in both animal and human research. Participants came to the lab on three consecutive days for an acquisition, an extinction, and a recall phase. During fear acquisition, two stimuli (CS+) were associated with a mild electrical shock, while two other stimuli (CS-) were never reinforced. During extinction, one CS+ and one CS- was always followed by a block of goal-directed eye movements. Blood Oxygenation Level-Dependent functional MRI data, pupil dilation, and skin conductance responses were recorded throughout all phases of the experiment. Preliminary results show that during eye movement blocks, there is suppression in the amygdala, hippocampus, and vmPFC relative to baseline compared to the fixation blocks. Further analyses will focus on whether this temporary suppression of brain regions supporting fear acquisition and extinction may affect retention of fear memories.

Disclosures: **L.D. De Voogd:** None. **J.W. Kanen:** None. **K. Roelofs:** None. **G. Fernández:** None. **E.J. Hermans:** None.

Poster

087. Emotion: Brain Imaging

Location: Hall A

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Program#/Poster#: 87.06/Y43

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant DGE0824162

Title: Dynamic shifts in large-scale brain network balance in response to acute stress

Authors: *C. B. YOUNG[1,2,3], G. RAZ[5], D. EVERAERD[2,3], C. F. BECKMANN[2,4], I. TENDOLKAR[2,3,6], T. HENDLER[7], G. FERNANDEZ[2,4], E. J. HERMANS[2,4];
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Psychiatry, [4]Dept. of Cognitive Neurosci., Radboud Univ. Med. Ctr., Nijmegen, Netherlands; [5]Dept. of Cognitive Neurosci., Maastricht Univ., Maastricht, Netherlands; [6]Dept. of Psychiatry and Psychotherapy, Univ. Hosp. Essen, Essen, Germany; [7]Departments of Physiol. and Pharmacol. and Psychiatry, Tel Aviv Univ., Tel Aviv, Israel

Abstract: The acute response to stress can be beneficial, such as during imminent threat, but also detrimental, for example when individuals are unable to regulate responses to minor stressors. Stressors trigger a cascade of neurochemical changes which are thought to result in alterations in coordinated activity across large-scale brain networks. However, how stress affects the dynamic interactions within and between such networks remains poorly understood. One recent theory states that as individuals exhibit a stress response, cohesion within the salience network (SN) increases, while the executive control network (ECN) is suppressed. To test this hypothesis, we used functional MRI and a mild stress induction paradigm (exposure to emotionally arousing cinematographic material), and examined how functional connectivity within and between the SN and ECN changes as participants react to this stressor. A 10 min movie fragment was first divided into a low-arousal and a high-arousal period based on subjective arousal ratings provided by a sample of 17 male participants. A separate sample of 120 male participants passively viewed the movie clip during an fMRI scan and heart rate data were simultaneously collected. Consistent with arousal ratings, heart rate was significantly higher during the high-arousal period in comparison to the low-arousal period of the movie, confirming the efficacy of the stress induction. To specifically address the question of how network interactions evolve over time when exposed to a stressor, we calculated heart rate and measured brain network cohesion in a dynamic manner within both movie periods. As predicted, we found that across time and within both periods, increased heart rate was accompanied by increased SN cohesion. Critically, during the high-arousal period only, the correlation between SN and ECN cohesion decreased as heart rate increased. Thus, exposure to acute stress disrupts coupling between the SN and ECN as individuals exhibit increasing physiological arousal. This finding demonstrates that acute stress is associated with a shift in brain function at the level of balance between large-scale networks. Such a global brain state change may explain shifts in cognitive functioning during acute stress and play an important role in the development and maintenance of stress-related mental disorders.

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Poster

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Department of Veterans Affairs, Rehabilitation Research & Development Service Career Development Award e7822w

Title: Altered resting state functional connectivity in the medial temporal lobe subsystem of the default mode network in posttraumatic stress disorder

Authors: *D. R. MILLER[1,3], S. M. HAYES[4,2,3], J. P. HAYES[5,2,4], J. M. SPIELBERG[4,2], G. LAFLECHE[3,2], M. VERFAELLIE[3,2];
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Abstract: Posttraumatic stress disorder (PTSD) is a psychiatric disorder that develops after exposure to life-threatening events and is characterized by debilitating re-experiencing, avoidance, and hyperarousal symptoms. Recent work has provided evidence for disrupted connectivity in intrinsic networks in PTSD, particularly the default mode network (DMN). In addition to two midline core hubs, the DMN consists of two functionally and anatomically distinct subsystems, a medial temporal lobe (MTL) system and a dorsal medial prefrontal cortex (dMPFC) system. The goal of this study was to examine the status of these subsystems in PTSD. PTSD is associated with impaired contextual fear extinction learning, and such learning depends on the ventral MPFC (vMPFC) and hippocampus, two regions that are part of the MTL subsystem of the DMN. Thus, we hypothesized that PTSD may differentially affect the MTL subsystem. Sixty-nine Operation Enduring Freedom/Operation Iraqi Freedom (OEF/OIF) veterans with PTSD and 44 OEF/OIF veterans without PTSD underwent two resting state fMRI scans with their eyes open. We assessed group differences in functional connectivity using a core hub (posterior cingulate cortex; PCC) as well as a node in each DMN subsystem (vMPFC and dMPFC) as seed regions. Results showed that individuals with PTSD had decreased functional connectivity between the PCC and the MTL subsystem, specifically the hippocampus and vMPFC. Groups did not differ in functional connectivity between the PCC and dMPFC subsystem. Connectivity between the PCC and hippocampus was negatively associated with total PTSD symptom severity and avoidance symptomatology. Using seed regions from the MTL and dMPFC subsystems, we found significantly reduced anti-correlation between the vMPFC node of the MTL subsystem and the dorsal anterior cingulate cortex in the PTSD group. There were no group differences in the dMPFC subsystem. These findings suggest that aberrant functional

connectivity within the DMN is restricted to the MTL subsystem in PTSD and may act as a mechanism by which contextual fear extinction processes are disrupted.

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Poster

087. Emotion: Brain Imaging

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 87.08/Z1

Topic: F.01. Human Cognition and Behavior

Title: Negatively connoted music increases brain activity in the prefrontal cortex: a nirs study

Authors: S. CROWEL, Z. MESSER, J. ADAY, C. BRATONIA, *M. ZIAT;
Psychology, Northern Michigan Univ., Marquette, MI

Abstract: In this study we examined changes in cortical hemodynamic activity using Near-Infrared Spectroscopy (NIRS) while participants were watching thirty second neutral video clips associated with a auditory music stimulus that can trigger a : 1) neutral, 2)positive (pleasant), or 3) negative (unpleasant) emotion. The main goal was to assess whether the three different conditions reflected changes of oxygenated (Hbo) and, deoxygenated (Hbr) in the prefrontal cortex while the videos were presented to participants. A NIRS probe (8 sources and 9 detectors) was attached to the front of the head using an elastic headband. The participants were also asked to rate the valence after each video on a scale from -2 to 2. NIRS data indicated an increase in HbO and HbR above baseline levels for the unpleasant condition as opposed to the pleasant and neutral conditions which showed decrease in HbO below baseline levels and no significant changes in HbR. These results suggest that when an unpleasant auditory stimulus is paired with a neutral video, the prefrontal cortex showed increased levels of HbO as well as HbR. These findings were also corroborated by the behavioral data, as the unpleasant condition was rated negatively (-1.33) when paired with a neutral video and was significantly different ($p < 0.01$) from the average rating for neutral and pleasant conditions that were rated +0.5 and +0.67 respectively.

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Poster

087. Emotion: Brain Imaging

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Program#/Poster#: 87.09/Z2

Topic: F.01. Human Cognition and Behavior

Support: ARC DP1093234

Title: A multivariate analysis of brain networks involved in facial gaze and emotion processing

Authors: *M. ZIAEI[1], W. VON HIPPEL[1], J. HENRY[1], N. EBNER[2], H. BURIANOVÁ[3]; [1]Univ. of Queensland, Brisbane, Australia; [2]Univ. of Florida, Gainesville, FL; [3]Ctr. for Advanced Imaging, Brisbane, Australia

Abstract: Eye gaze direction plays a fundamental role in social interaction in that it influences the perception of facial features and particularly the processing of facial emotion expressions. Yet, the neural mechanisms that underlie the integration of gaze and emotional cues in faces are not well understood. Given the complexity of the involved higher cognitive processes, it is reasonable to assume that large-scale brain networks are recruited, rather than discrete brain regions. The objective of this study was to delineate the brain networks that subserve the processing of emotional expressions with direct and averted gaze. Twenty healthy adults participated in a functional magnetic resonance imaging experiment in which they were asked to identify the emotional expressions of happy, angry, or neutral faces, displayed with direct or averted gaze. The results showed that the functional networks engaged during the identification of angry faces were different depending on the gaze, whereas the same functional network was recruited for direct and averted happy faces. Angry averted gaze recruited a network that comprised bilateral amygdala, striatal nucleus, and superior temporal gyrus, whereas angry direct gaze recruited bilateral insula and anterior cingulate gyrus, the critical nodes of the salience network. Our findings provide novel insights into the impact of eye gaze on processing of angry emotional expressions, and the results have broader implication for understanding the underlying neural mechanisms of social-cognitive functions involved in the processing cues from the faces in healthy and clinical populations.

Disclosures: **M. Ziaei:** None. **W. von Hippel:** None. **J. Henry:** None. **N. Ebner:** None. **H. Burianová:** None.

Poster

087. Emotion: Brain Imaging

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Program#/Poster#: 87.10/Z3

Topic: F.01. Human Cognition and Behavior

Title: Differences in resting-state functional connectivity 24-hours after delay and trace fear conditioning

Authors: *L. HOPKINS[1], D. H. SCHULTZ[2], F. J. HELMSTETTER[1];
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Abstract: Resting-state functional connectivity (RSFC) measures synchronous activity between brain areas when participants are not engaged in a task. RSFC can be used to investigate connectivity profiles after learning when memory is being consolidated. Here we explored differences in functional connectivity at rest after a delay (DFC) or trace (TFC) fear conditioning task. In each procedure a neutral visual stimulus signaled the occurrence of shock. In the DFC group the CS and UCS overlap and co-terminate while in TFC the CS and UCS are separated by a blank period of time called the trace interval. It is well established that DFC and TFC rely on different brain areas during acquisition. The amygdala is involved in both but TFC engages additional structures including the hippocampus and medial prefrontal cortex (mPFC). As stable long term memory forms after training, some brain areas are engaged time-dependently while others are always required. Most work on RSFC and memory is restricted to the period immediately after learning. Here subjects trained with DFC or TFC underwent an 8-minute resting state scan both immediately after conditioning and 24-hours later. After parcellation into multiple ROIs we compared RSFC between groups at the 24-hour time-point. We also compared RSFC within each group across time. Across groups, increased RSFC was apparent in the TFC group relative to the DFC group in two ROI pairs 24-hours later. RSFC was enhanced between the temporal pole and insula as well as between the posterior cingulate (PCC) and cerebellum. Across time, DFC subjects had enhanced RSFC between one ROI pair of temporal lobe structures 24-hours later whereas TFC subjects had enhanced RSFC between two ROI pairs involving temporal, frontal, and parietal structures 24-hours later. The enhanced connectivity between more widespread brain areas involved in emotional processing, complex cognition, and memory in the TFC group 24-hours post-conditioning may reflect the additional changes at the systems level required for the long-term consolidation of a more complex fear memory. These results indicate that RSFC can be used to identify more long-term brain changes that may reflect the memory consolidation process and that may be unique to specific forms of learning.

Disclosures: L. Hopkins: None. **D.H. Schultz:** None. **F.J. Helmstetter:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: Medical Research Council (G0100102)

Dr Mortimer and Theresa Sackler Foundation

Dame Stephanie Shirley

NordicNeuroLab

Title: Imaging of social cognition in low-functioning autism

Authors: *A. G. MCKECHANIE[1,2], T. J. MOORHEAD[1], C. THORBURN[2], N. ROBERTS[1], E. C. JOHNSTONE[1], D. G. C. OWENS[1], A. C. STANFIELD[2];
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Abstract: Background: A major objective of autism spectrum disorders (ASD) research has been to delineate the affected neural systems. Functional magnetic resonance imaging (fMRI) of the brain has played a key role in this research, but its application has been limited to affected individuals with preserved or superior intellectual functioning due to difficulties in obtaining informed consent, tolerability of the scanner environment and the lack of suitable fMRI tasks for individuals with intellectual impairment. Objective: The objective of this study was to demonstrate that it is feasible to reliably use a fMRI task in individuals with intellectual impairment ± co-morbid autism spectrum disorder (ASD) in a task of implicit emotion processing, and to explore the neural correlates of emotion processing in individuals with comorbid intellectual impairment and autism spectrum disorder. Methods: We conducted an fMRI study using an implicit facial emotion processing task. Diagnosis of ASD was confirmed using the Autism Diagnostic Observation Schedule (ADOS). Prior to undergoing the scan, participants were able to practice the task on a laptop and also to practice on a mock scanner (where there were no time pressures). Results: All participants (N=18) successfully completed the functional task, thus demonstrating that fMRI techniques are possible to use in those with impaired intellect ± comorbid autism spectrum disorder. When examining the differential neural response to the fearful faces with the response to the neutral faces, there were a number of brain regions which showed significantly greater activation in the non-ASD group, compared to the ASD group. These were in similar areas as described in the broader ASD literature. In contrast, there were no regions of increased activation in the ASD group. Conclusions: In this study we

have demonstrated that it is possible to undertake fMRI studies in individuals with intellectual impairment, who were previously considered unable to participate in such studies. We have mirrored findings of decreased cerebral function in brain regions previously linked with emotional processing deficits in non-intellectually impaired individuals with ASD.

Disclosures: A.G. McKechanie: None. **T.J. Moorhead:** None. **C. Thorburn:** None. **N. Roberts:** None. **E.C. Johnstone:** None. **D.G.C. Owens:** None. **A.C. Stanfield:** None.

Poster

087. Emotion: Brain Imaging

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 87.12/Z5

Topic: F.01. Human Cognition and Behavior

Support: NIMH BRAINS: R01MH091864

NSF GRFP: 2014177271

Title: The effects of early adversity on emotional appraisal: implications for amygdala-mPFC circuit development

Authors: *M. R. VANTIEGHEM[1], L. GABARD-DURNAM[1], J. FLANNERY[2], B. GOFF[3], D. GEE[4], K. HUMPHREYS[5], E. TELZER[6], C. CALDERA[3], T. HARE[7], N. TOTTENHAM[1]; [1]Columbia Univ., New York, NY; [2]Univ. of Oregon, Eugene, OR; [3]Univ. of California Los Angeles, Los Angeles, CA; [4]Weill Cornell Med. Sch., New York, NY; [5]Tulane Univ. Sch. of Med., New Orleans, LA; [6]Univ. of Illinois at Urbana-Champaign, Champaign, IL; [7]Univ. of Zurich, Zurich, Switzerland

Abstract: Previous research suggests that early adverse caregiving alters the developmental trajectory of amygdala-prefrontal circuitry, resulting in heightened emotional reactivity and adult-like fear learning. This research has focused primarily on the function of this circuit when processing facial expressions of clear valance, such as those of fear and happiness. However, adult studies have shown that facial expressions with ambiguous affect (i.e., surprised) can elucidate trait-like emotional biases that are associated with amygdala-mPFC circuitry. Further, typically developing children show a negative bias to surprise faces that declines with age. The current study examined the effects of early adversity on emotional appraisals of surprised faces in a sample of previously institutionalized (PI) youth and comparisons between the ages of 6-14 (N=167, mean age=9.8, M=65). Participants viewed a standardized set of surprised, happy, and

angry faces and rated whether each face “felt good or felt bad” as quickly as possible. Resting-state fMRI scans were acquired for a subset of participants to examine the functional integrity of amygdala-mPFC circuitry. PI youth showed more positive (i.e. adult-like) appraisal of surprised faces relative to comparisons ($p<0.05$). Preliminary findings suggest that stronger (i.e. more mature) amygdala-mPFC connectivity was associated with more positive appraisal of surprised faces across both groups ($p<0.05$). Although PI youth showed elevated levels of social anxiety at the group level, those individuals with more positive appraisals showed lower levels of social anxiety ($p<0.01$). These findings suggest that more positive appraisal of ambiguous emotional cues may reflect an ontogenetic adaptation following early adversity.

Disclosures: M.R. Vantieghem: None. **L. Gabard-Durnam:** None. **J. Flannery:** None. **B. Goff:** None. **D. Gee:** None. **K. Humphreys:** None. **E. Telzer:** None. **C. Caldera:** None. **T. Hare:** None. **N. Tottenham:** None.

Poster

087. Emotion: Brain Imaging

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 87.13/Z6

Topic: F.01. Human Cognition and Behavior

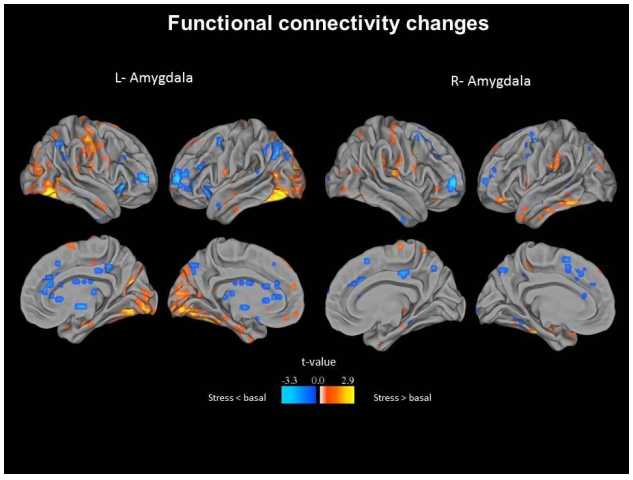
Support: DGAPA-UNAM

Title: Resting state conectivity of the amygdala after the exposure to a stressful video

Authors: *D. VÁZQUEZ CARRILLO[1], J. MARTÍNEZ-SOTO[2], S. ALCAUTER[1], E. PASAYE[1], L. GONZALES-SANTOS[1], F. A. BARRIOS[1];
[1]Inst. de Neurobiología, Univ. Nacional Autónoma De México (UNAM), Queretaro, Mexico; [2]Univ. de Guanajuato, Guanajuato, Mexico

Abstract: Introduction: Everyday we are exposed to a variety of stressful conditions. Since stress has a negative impact in emotional and physical health, the study of stress response and its functional basis in human brain is of great interest for the neuroscience and health-care community. Amygdala has long been implicated as a main responsible in the triggering of brain's response to stressful conditions [Veer, *et. al.*2011]. The aim of this study is to characterize the change in amigdalae's resting state functional connectivityafter been exposed to a stressful event. MethodologyResting state fMRI and high resolution T1 images were acquired in 32 healthy male participants (mean age: 36.18 and sd= 12.46), before (basal) and after (stress) being exposed to a stressful video [extracted from Faces of Death #4]. Stress was quantified with the Stress and

Activation Adjectives Checklist (Martínez-Soto, *et. al.*2012). The left and right amygdalae were segmented on the T1 images for each individual using FSL's FIRST. After standard preprocessing (no smoothing, no global signal regression), functional connectivity maps were obtained using each amygdala as seed. Paired t-tests were performed to identify significant changes in connectivity between the basal and stress states. Results: Subjects weresignificantly morestressedafter watching the video ($t(32)=-3.65$, $p<0.01$). Decreased functional connectivity($p<0.05$, uncorrected)was evident in regionsinvolved in cognitiveprocessingand effortful regulation of affect, such as superior frontal gyrus, middle frontal gyrus, PCC, and precuneus(Figure).Increased connectivity ($p<0.05$, uncorrected)was evident in occipitalandinferior temporalcortices, as well as in pre-and post-central sulci. Functional connectivity changes seem to be more dramaticfor the left amygdala(Figure). Conclusion: Subjects were significantlymore stressed after watching the video. Amygdala's functional connectivity showed significant changes with frontal and posterior brain regions, relevant for both emotional and cognitive processing.Betterunderstanding of brain stress responsemay play a crucial role in improving



Disclosures: **D. Vázquez Carrillo:** None. **J. Martínez-Soto:** None. **S. Alcauter:** None. **E. Pasaye:** None. **L. Gonzales-Santos:** None. **F.A. Barrios:** None.

Poster

087. Emotion: Brain Imaging

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Program#/Poster#: 87.14/Z7

Topic: F.01. Human Cognition and Behavior

Title: Temporal dynamics of fear learning and generalization in the human brain

Authors: *S. NASR[1], E. A. BOEKE[2], S. N. DECROSS[2], R. P. F. WOLTHUSEN[2], R. B. H. TOOTELL[2], M. R. MILAD[2], D. J. HOLT[2];
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Abstract: Functional MRI studies have shown that an extensive network of regions, including the amygdala, hippocampus, insula, anterior cingulate cortex, and caudate nucleus, show conditioned fear responses in humans. However, it remains unclear which of these regions contributes to the acquisition versus the expression of conditioned fear. To examine this question, we conducted an fMRI study in which 37 healthy humans underwent a Pavlovian fear conditioning and generalization procedure. Skin conductance responses (SCR) and fMRI data were collected simultaneously throughout the experiment. During the conditioning phase, a face stimulus was intermittently paired (CS+) or not (CS-) with a mild electrical shock delivered to the fingers. During the subsequent generalization phase, 5 generalization stimuli (m1-m5, individualized based on each subject's discrimination threshold) and the CS+ and CS-, were presented. Four time intervals during conditioning and two time intervals during generalization (two trials per interval) were examined. An anatomical region-of-interest fMRI analysis was conducted. Fear generalization was defined as a response to the generalization stimulus that was most similar to the CS+ (m1) that was significantly greater than the response to the CS-. Based on the SCRs, the subjects acquired differential conditioned fear responses and showed fear generalization. In the fMRI data, during fear conditioning, ANOVAs revealed a significant stimulus type by time interaction for the anterior insula, caudate nucleus and hippocampus, due to a progressive increase in differentiation in the BOLD responses to the CS+ vs. CS- as learning occurred (CS+ > CS- in the anterior insula and caudate nucleus; CS- > CS+ in the hippocampus). The anterior insula, and to a lesser extent the anterior cingulate cortex, also showed significant generalization of fear responses, that was sustained over time. In summary, we found that the anterior insula is involved in both the acquisition and expression (as manifested by fear generalization) of conditioned fear responses in humans, suggesting that interoceptive processing is critically involved in both learning and retrieval of fear signals in humans. Moreover, the caudate nucleus and hippocampus showed evidence for a role in the acquisition of fear and safety signals, respectively, but not in generalization. Thus, these two regions may be primarily involved in communicating learned fear and safety signals to other structures, such as the prefrontal cortex.

Disclosures: S. Nasr: None. **E.A. Boeke:** None. **S.N. DeCross:** None. **R.P.F. Wolthusen:** None. **R.B.H. Tootell:** None. **M.R. Milad:** None. **D.J. Holt:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: KAKENHI

JST SAKIGAKE

Title: Dissociable Neural correlates of positive and negative emotions during human sound processing

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Abstract: What kind of mechanism in the brain yields positive or negative emotion while we process sounds? Although there are some studies investigating how brain activity changes depending on genres and likability of music, few studies have contrasted positive and negative emotions by comparing positive and negative sounds. Here, we investigate how the positive and negative emotions arise by conducting a functional magnetic resonance imaging experiment of sound evaluation. Participants (n=26) were instructed to bring their own favorite music, and we prepared nine different noise sounds ranging from machine noise to mosquito flying sound. Participants were exposed to three different blocks (i.e, music, noise, and nothing: order was randomized), each of which lasted for 30s. Each kind of block appeared 13 times in a session and the total time of the session was 20min, and all participants experienced two sessions. Participants were asked to evaluate each block by five different grades (Most comfortable, A little comfortable, Neutral, A little uncomfortable, Most uncomfortable) by pressing a button. We also measured heartbeat, breathing, pulse wave, and skin conductance of each participant during MR scanning. Functional image processing were carried out using the Statistical Parametric Mapping software (SPM12, London) on a MATLAB (The MathWorks, MA) platform, and we also conducted a correlation analysis of beta values obtained from the first level analysis of SPM with each participant's average evaluation in each condition. We found significant increase of activity during the favorite music block in comparison with the noise block in the nucleus accumbens (NAcc), anterior cingulate cortex (ACC), and auditory cortex ($p<.001$, unc.). On the other hand, we found significant increase of activity during the noise block increased in the amygdala, hippocampus, and dorsolateral prefrontal cortex (DLPFC) ($p<.001$, unc.). In addition, beta values in NAcc and ACC were correlated with each subject's average evaluation during the music condition, and the beta values in the amygdala and auditory cortex were correlated with each subject's average evaluation during the noise condition. Interestingly, we also found that

noise activity in the amygdala was weaker in the second session, while the one in DLPFC was higher in the second session, indicating some opposite contributions of these two regions. These results indicate that different regions in the brain are involved in the generation of positive and negative emotions during human sound processing. It is an important future research topic to clarify what network mechanism is the key to the generation of subjective sensations.

Disclosures: **N. Okamoto:** None. **M. Haruno:** None.

Poster

087. Emotion: Brain Imaging

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Topic: F.01. Human Cognition and Behavior

Support: NSERC

Title: Neural specificity in processing visual and auditory socially-relevant information as revealed by fMRI

Authors: ***J. WHITEHEAD**[1,3,4], J. L. ARMONY[2,3,4];
[1]Integrated Program in Neurosci., [2]Psychiatry, McGill Univ., Montreal, QC, Canada; [3]Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada; [4]BRAMS Laboratory, Ctr. for Res. on Brain, Music and Language, Montreal, QC, Canada

Abstract: Social communication in a natural setting relies on input from various sensory modalities. Some emotion-sensitive brain regions, such as the amygdala, can respond to relevant information conveyed in different modalities (e.g., auditory and visual) using a variety of channels (e.g., face and body expressions, speech and music). Yet, it remains largely unknown whether these very different types of stimuli are processed by the same neural populations, or whether the observed responses, as seen with techniques such as functional magnetic resonance imaging (fMRI), reflect the engagement of independent yet overlapping uni-modal, category-specific neurons. To directly address this question, we employed an fMRI adaptation paradigm in a series of experiments designed to measure neural responses to social communicative signals as a function of their sensory modality (visual vs. auditory), affective value (neutral vs. fear) and category (music vs. speech; faces vs. body expressions). We used a fast (TR=529ms), high-resolution (8 mm3 isotropic) multiband sequence to maximize temporal and spatial specificity of the observed responses, as well as statistical power. Initial results confirmed modality- and category-specific adaptation effects in cortical regions, which could not be explained solely in

terms of category differences along basic physical properties. Our findings for music, a powerful emotionally arousing stimulus with no obvious survival or evolutionary relevance, are particularly interesting as they can provide new empirical evidence that can inform the ongoing debate about the nature of the neural representation of such type of stimulus class.

Disclosures: J. Whitehead: None. **J.L. Armony:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant DA023248

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NIH Grant AA021449

Title: A meta-analytic review of the role of ventromedial prefrontal cortex and amygdala in emotion regulation

Authors: *S.-J. TSAI[1], C.-S. R. LI[2];
[1]Med., Natl. Yang-Ming Univ., Kaohsiung City, Taiwan; [2]Psychiatry and Neurobio., Yale Univ., New Haven, CT

Abstract: Background: The ventromedial prefrontal cortex (vmPFC) has long been implicated in regulating fear response. Recent literature points to differential responses of subregions of the vmPFC to positive and negative affect (Myers-Schulz B et al, 2012 Mol Psychiatry). Therefore, a detailed examination is needed to understand how the vmPFC interacts with amygdala under various emotional contexts. **Methods:** Human fMRI studies are searched on PubMed with keywords of [(ventral medial prefrontal cortex, ventromedial prefrontal cortex, OR vmPFC) AND (amygdala)]. Studies are included if performed on healthy subjects with significant same- or opposite- direction of activities reported for both the vmPFC and amygdala. Emotional contexts in these studies are further categorized as passive (e.g., viewing pictures of facial expressions) and participating (e.g., emotional control and appraisal; cost-effect/moral judgments), with subcategories of emotions (e.g., happy, sad, angry, and fear) specified. We performed neuroanatomical meta-analysis of activity patterns by activation likelihood estimation (ALE) (Eickhoff SB et al, 2009 Hum Brain Mapp). **Results:** The vmPFC and amygdala co-

activate to passive happy, angry and sad emotion induction, but not fear, which evoked increased amygdala and decreased vmPFC activity. An opposing pattern of vmPFC and amygdala activity is shown under emotional control and reappraisal. vmPFC activations in association with amygdala activity changes are mapped to functionally distinct subregions in general agreement to the account of perigenual vmPFC response to positive emotion. Elevated activity of perigenual vmPFC and amygdala were also noted when subjects participate in ethical decisions or risk-seeking behaviors. **Conclusions:** These preliminary results echo recent proposal of separate functional subregions of the vmPFC, and suggest the need of more research on the role of vmPFC subregions in emotional regulation and decision making.

Disclosures: S. Tsai: None. **C.R. Li:** None.

Poster

087. Emotion: Brain Imaging

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Topic: F.03. Motivation and Emotion

Support: NRF-2014R1A1A2059849

NRF-2012H1A2A1017177

Title: The effect of dynamic facial expressions on subsequent emotional information processing: an fMRI study

Authors: *S. KIM, H. YOON, S. KIM;
Korea Univ., Seoul, Korea, Republic of

Abstract: Responses to emotional information are known to be modulated by regulatory efforts. However, little is known as to how the contextual information influences experiential and neural correlates of emotional information processing, especially when the context is emotionally conflicted with the valence of ongoing emotional information. We wanted to examine in what way preceding dynamic facial expressions of emotions modulate neural to subsequent unpleasant information. Twenty-three healthy adults participated in this functional neuroimaging study (12 women, mean age = 22.83, SD = 2.26). Inside the brain scanner, participants were presented with a series of negative and neutral pictures and their task was to rate the level of emotional arousal elicited by each picture. Each trial of the task started with the presentation of one of the dynamic facial expression that lasted for 2 s: happy, neutral, and angry expression. Then, a crosshair

fixation appeared for about 2 s with jittered durations, followed by either negative or neutral pictures for 6 s. Then, participants rated the level of emotional arousal on a Likert scale ranging from 1 to 4 for 3 s. Brain images were acquired by a 3T Siemens scanner located at Korea University Brain Imaging Center and analyzed using SPM8. Regions of interest (ROIs) were functionally defined as spheres with 6mm radius centered at the peaks of activation clusters identified from the overall contrast of negative versus neutral pictures. A one-way ANOVA on percent signal changes of each ROI was conducted with a factor of facial expressions. The cuneus showed the main effect of facial expression with a linearly increasing activity trend from positive to negative face conditions. Correlation analysis revealed that the activation level of cuneus was negatively correlated with that of amygdala when the picture was preceded by positive face. We also found significant negative correlation of cuneus activations with vlPFC activations in the happy face condition. These results indicate that the emotional context which is inconsistent with subsequent emotional information gives rise to conflicting situation that causes attenuated cognitive processing of information. This might make the emotional response greater but allow one to attempt to resolve the conflict

Disclosures: **S. Kim:** None. **H. Yoon:** None. **S. Kim:** None.

Poster

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Topic: F.03. Motivation and Emotion

Title: Working memory load enhanced interpersonal reconnection

Authors: ***K. SAKAKI**[1], T. NOZAWA[1], R. YOKOYAMA[2], Y. SASAKI[1], R. KAWASHIMA[1]; [1]Tohoku Univ. (IDAC), Aoba-Ku Sendai-Shi Miyagi-Ken, Japan; [2]Kobe Univ., Chuo-Ku, Kobe-Shi, Hyogo-Ken, Japan

Abstract: Some previous studies showed that response for social exclusion had individual difference. One is the antisocial response such as withdrawal or aggressive behavior. The other is the prosocial response such as trying to make new friendships. This phenomenon is called “interpersonal reconnection”. Our previous fMRI study revealed that people who had lower reconnection tendency (RT) showed higher insula activity, which is known to relate to negative emotions, during imaging social situation after exclusion. We hypothesized that working memory load, which is known to distract emotion, could suppress the insula activity and negative emotions and promote reconnection. This study was approved by the Ethics Committee of

Tohoku University Graduate School of Medicine. Written consent was obtained from all subjects. Two runs of fMRI scans were conducted on 18 university students. Each run contained two tasks: a Cyberball task (CB) and a Reconnection evaluation task with working memory load (RETWM). CB is a virtual ball-tossing task. We set up 2 conditions: an Inclusion condition (I) that is a control condition in which the probability of receiving a ball was set to 0.5, and an Exclusion condition (E) in which the probability was set to 0.1. A trial of RETWM consisted of 4 phases. In the first phase, 2 figures were presented randomly on 2 corners of the screen. In the second phase, subjects imagined presented situation as clearly as possible, and rated their desire to participate in the third phase. In the last phase, subjects answered the location of a specified figure. In the control condition of RETWM, the answer was visible throughout the 4 phases. The trial was repeated 8 times per session. We conducted 4 sessions for each subject, corresponding to the 2 by 2 task design of E or I and Memory (M) or Control (C). We defined the RT for each subject as the [EC - IC] and the promotion effect of WM load on reconnection (WM-effect) as the [(EM - EC) - (IM - IC)] in the averages of the attractiveness-corrected ratings. Behavioral result suggested that the promotion effect of WM load may be effective only for people who have low RT. SPM analysis showed that (1) right dorsolateral prefrontal cortex (DLPFC) was more activated getting a working memory load on thinking about social situation after exclusion; and (2) the activation of primary visual cortex was negatively correlated with WM-effect. It is known that the DLPFC is related to emotion regulation, and vividness of mental imagery was correlated with activation in the primary visual cortex. Our results suggested that WM load (1) promoted emotion regulation; and (2) inhibited vivid imaging, leading to promotion of reconnection.

Disclosures: **K. Sakaki:** None. **T. Nozawa:** None. **R. Yokoyama:** None. **Y. Sasaki:** None. **R. Kawashima:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: FNS Grant FN 105314-140522

National Center of Competence in Research “Affective Sciences – Emotions in Individual Behaviour and Social Processes”

Title: Distinct contributions of acoustic parameters in the recognition of emotional prosody

Authors: *D. BENIS, J. PERON, T. OTT, S. FRÜHHOLZ, D. GRANDJEAN;
Neurosci. of Emotion and Affective Dynamics Lab., Univ. of Geneva, Geneve, Switzerland

Abstract: Emotional prosody is defined as modifications in segmental and supra-segmental speech parameters during an emotional episode (Grandjean, Banziger, & Scherer, 2006). Relevant acoustic parameters for emotional prosody recognition include fundamental frequency (f0), tempo, amplitude, and repartition of spectral power across frequency bands. While emotional prosody recognition have been shown to correlate with perceived modulations of these acoustic features (Schirmer and Kotz, 2006; Sauter et al., 2010), the distinct contribution of acoustic cues to emotion recognition performance remains unclear. Furthermore, while emotional prosody has been shown to involve the basal ganglia network (Frühholz et al., 2014), distinct contributions of acoustic cues on basal ganglia activity during emotional prosody decoding remains to be investigated. The aim of the present study was then to investigate the influence of two acoustic features (namely f0 and amplitude) on emotional prosody recognition performances and on basal ganglia BOLD activity. To this end, we analyzed behavioral performances and BOLD responses in the basal ganglia of 22 healthy participants, while they performed an emotional vocal stimulus evaluation task (Péron, Grandjean, et al., 2010). In this experiment, three types of emotional prosodic stimuli were presented: 1. Original vocal stimuli; 2. F0 flattened stimuli and 3. Energy flattened stimuli. Participants were then instructed to judge the emotional content of each stimulus using a set of visual analog scales (one scale for each emotion presented: happiness, neutral, anger, disgust, fear) as well as the arousal level resulting from stimulus presentation. We observed a global accuracy decrease on emotional scales as well as a decrease in arousal reports during classification of emotional f0 flattened stimuli compared to native stimuli. These effects were significantly more important for anger compared to happiness stimuli, and resulted in an increase of classifications on the non-target neutral scale. Interestingly, such behavioral effects were not observed for energy flattened emotional stimuli. F0 flattening was also associated with modulations of BOLD activity in the basal ganglia system during emotional prosody decoding, Taken together, these results seem to confirm the importance of the fundamental frequency in emotional prosody recognition and suggest that acoustic cues are used differentially according to the valence of the prosodic stimulus processed, through differential activations of the basal ganglia system.

Disclosures: D. Benis: None. **J. Peron:** None. **T. Ott:** None. **S. Frühholz:** None. **D. Grandjean:** None.

Poster

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Wheaton College Alumni Association Faculty Grant Program

Title: Behavioral and ERP responses to emotionally evocative images among high and low trait anger college age men

Authors: K. ROSS[1], D. ADDLEMAN[2], A. EARLY[1], C. REYES[1], S. BAKER[1], H. O'HORA[1], R. E. PHINNEY[2], W. M. STRUTHERS[2], H. WHITNEY[3], *N. THOM[4,1];
[1]Applied Hlth. Sci., [2]Psychology, [3]Physics, [4]Wheaton Col., Wheaton, IL

Abstract: Little is known about how specific traits, such as trait anger, alter emotional processing. Gaining a better understanding of how traits alter emotional processing could improve clinical care. Therefore, we tested the effect of trait anger on behavioral and EEG responses to a variety of emotionally evocative scenes. Men ages 18-22 with either normal levels of self-reported trait anger (STAXI = 18.4 +/- 5.4; n=16) or high levels of trait anger (STAXI = 28.2 +/- 2.2; n=11) were recruited to examine the effects of emotionally evocative scenes previously shown to elicit anger, fear, and joy (Harmon-Jones, 2006 & 2007; Mikels, 2005)). During picture viewing, event-related potentials (EPN and LPP) and arousal, valence, and anger intensity were measured. Mixed-model ANOVA with follow-up contrasts were used to detect differences between/within dependent variables. The main novel finding is that men who reported high levels of trait anger also reported higher levels of anger intensity when viewing fear-inducing pictures ($p=.007$). As expected, all men reported greater arousal when viewing evocative pictures ($p < 0.001$). Follow-up analysis revealed higher arousal ratings ($p<0.05$) for joyful, fearful, and anger scenes compared to neutral scenes. In addition, all men viewed the anger and fearful pictures as unpleasant and the joyful pictures as pleasant ($p < 0.001$). Follow-up comparisons indicated that all pictures types were rated differently from each other on valence (all ps < 0.001). Among men with normal trait anger, EPN amplitudes were greatest for joy and fearful images (ps < 0.03) and all emotionally evocative pictures showed greater amplitude compared to neutral images ($p = 0.04$). For the LPP, mean amplitude was not different between anger, fear, and joy images (all ps > 0.05), but LPP amplitude across all emotion types was elevated when compared to neutral pictures ($p = 0.008$). ERP amplitudes have only been analyzed for men with low trait anger; analysis of high trait anger men and the comparison between the two groups is forthcoming. These findings present novel results examining how trait anger affects behavioral and neuro-electric responses to emotionally evocative picture stimuli. Because men with high trait anger became more angry in response to fearful or threatening images, these results are consistent with the elicitation hypothesis (Deffenbacher et al., 1996; Quinn et al., 2014) which suggests that high trait anger elicits anger more frequently and due to a

broader range of stimuli. Future studies should more robustly examine how trait anger triggers response to various emotionally laden stimuli, such as sadness or sorrow.

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Poster

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NIMDS 1R01MH105397-01

Leon Levy Center for Mind, Brain & Behavior

NYSCF-Robertson Neuroscience Investigator Award

Title: Neural mechanisms of communication via facial expression

Authors: *S. V. SHEPHERD, W. A. FREIWALD;
The Rockefeller Univ., New York, NY

Abstract: Human communication abilities are unique, but build upon a common foundation we share with our primate kin. Primates live in complex social groups with which they must coordinate their behavior, exchanging signals comprising postures, movements, and vocalizations. Scientists have made progress in understanding how facial signals are decoded in primate brains, but know almost nothing about how these signals are produced. We recorded brain activity in monkeys seeing and responding to communicative signals in an MRI scanner. Stimuli were made from video recordings of six monkeys producing a variety of facial expressions. Stimulus videos were simultaneously recorded from multiple angles; a half-silvered mirror was used when recording direct gaze to facilitate naturalistic eye contact. Two 11-second clips were gathered from each monkey, synchronously from three viewpoints. Phase scrambled versions of each video were used as low-level visual controls, and were generated by randomly rotating each Fourier component a consistent amount across each frame. To reduce habituation, we limited exposure to each video. Long (13 second) gaps were placed between videos. Within

each run, only one video per subject was shown. Within each session, each video (i.e. subject, event, perspective and scramble) was shown exactly once. Monkeys produced an affiliative gesture, called a 'lipsmack', in response to brief video of subject-directed monkey expressions. Facial movements were recorded by MR-safe video camera and later analyzed both digitally and by manually scoring behavior while blind to stimulus condition. IRON-fMRI imaging was used to track changes in cerebral blood volume associated with neural activity. By comparing both perceived and produced facial behavior to these simultaneously-recorded brain images, we identified key regions involved in processing social interactions and translating perceived signals into appropriate expressive responses. We find that perception of social stimuli activated the extended face patch system, and that production of the macaque 'lipsmack' signal correlated with activation of facial motor regions. Finally, we examine functional correlations between perceptual and motor face patches to determine candidate pathways by which these socially-driven facial expression may arise.

Disclosures: S.V. Shepherd: None. **W.A. Freiwald:** None.

Poster

087. Emotion: Brain Imaging

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 87.23/Z16

Topic: F.02. Animal Cognition and Behavior

Title: Early social experience influences cortical gray matter integrity in chimpanzees (Pan troglodytes): A sourced-based morphometry analysis

Authors: *K. R. DAVIDEK[1], W. D. HOPKINS[2];
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Abstract: Although recent work has indicated the influence of early social experiences on behavioral and cognitive development in chimpanzees (Pan troglodytes), much less is known regarding their impact on overall cortical development. Here, we used source-based morphometry (SBM) to quantify differences in structural gray matter connectivity in 92 adult chimpanzees matched on age, sex and scanner magnet who were either mother-reared (MR) or human-reared (HR). The original SBM analysis identified 28 independent component regions. Multiple analyses of variance revealed that MR and HR chimpanzees differed in eight component regions which included the cerebellum, dorsal prefrontal cortex, anterior cingulate, superior and middle temporal gyri, and the precentral inferior gyrus. For all but two components, MR chimpanzees had significantly higher weighted component scores compared to HR

individuals. Our results demonstrate the long-lasting impacts of early social experience on cortical gray matter development and morphology in regions critical for emotional and cognitive processes.

Disclosures: K.R. Davidek: None. **W.D. Hopkins:** None.

Poster

088. Emotion: Information Processing

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 88.01/Z17

Topic: F.03. Motivation and Emotion

Title: Electroencephalographic correlation during visual, maternal and erotic stimulation in mothers at different stages of postpartum

Authors: *M. PÉREZ-HERNÁNDEZ[1], R. M. HIDALGO-AGUIRRE[2], M. HERNÁNDEZ-GONZÁLEZ[2], C. AMEZCUA[2], M. A. GUEVARA[2];
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Abstract: The postpartum period is a critical reproductive state in which women present several hormonal, neural, behavioral and emotional changes. The hormonal changes underlying the onset of lactation, have been associated with the persistent inhibition of ovaric cycle, which, mainly in primiparous mothers, are associated with a diminished libido and less interest for those stimuli not related to the newborn. The aim of this study was characterize the prefrontal-parietal electroencephalographic correlation (rEEG) of primiparous mothers at different stages of postpartum during visual, maternal and erotic stimulation. In this study participated primiparous nursing mothers that were divided in three groups postpartum (PPT): Nursing 1 (N1, 1.5 to 3 PPT months), Nursing 2 (N2, 4 to 5.5 PPT months) and Nursing 3 (N3, 6.5 to 8 PPT months). A fourth group conformed by no mothers (NM) was considered as a control group. The EEG was recorded on prefrontal and parietal areas during observation of the maternal and erotic videos. The mothers of the N1 group showed a lower rEEG intrafrontal left (F1-F3) as well as a lower intrahemispheric prefrontal-parietal rEEG (F3-P3, F4-P4) only during the observation of erotic stimuli. Groups N2 and N3 showed changes during the observation of both stimuli; when they watched the erotic video showed a lower rEEG intrahemispheric prefrontal-parietal left (F3-P3) and when they watched the maternal video showed a lower rEEG interhemispheric prefrontal (F3-F4) and intrahemispheric prefrontal left rEEG (F1-F3). This data suggests that the degree of coupling among prefrontal and prefrontal-parietal cortices changes in relation to different stages

of postpartum. These results provide information about the brain functional changes experienced by the nursing mothers through the postpartum, associated to the processing of maternal and sexually relevant stimulus.

Disclosures: **M. Pérez-Hernández:** None. **R.M. Hidalgo-Aguirre:** None. **M. Hernández-González:** None. **C. Amezcua:** None. **M.A. Guevara:** None.

Poster

088. Emotion: Information Processing

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 88.02/Z18

Topic: F.03. Motivation and Emotion

Title: Evoked neural harmonics during naturalistic viewing: dissociable effects of perceptual quality and hedonic content

Authors: ***V. MISKOVIC**, K. KUNTZELMAN, N. HANSEN, N. FLETCHER; Psychology, Binghamton Univ., Binghamton, NY

Abstract: Population-level neuronal responses in early visual cortex can be driven by the periodic presentation (ON/OFF flicker or counter-phase modulation) of a stimulus at a fixed frequency. This type of periodic stimulus delivery elicits large-scale oscillatory field potentials (known as steady state visual evoked potentials or SSVEPs) with multiple frequency components. In addition to the neuronal responses that match the input frequency of the driving stimulus (frequency following or 1F evoked potentials), there are also robust responses at the second harmonic (frequency doubling or 2F components). Frequency following and frequency doubling neuronal responses appear to reflect the contributions of distinct types of cells in the visual cortex with different functional roles. Preliminary evidence employing simple visual cues suggests that frequency following responses provide a high fidelity representation of the sensory qualities of the stimulus (contrast, luminance, coherence) while frequency doubling neurons exhibit substantial modulation by attention (responses are amplified for features with high behavioral relevance). We tested this hypothesis concerning the differential roles of frequency following and frequency doubling visual responses by recording SSVEPs to naturalistic scenes flickered ON/OFF at 12 Hz. We systematically varied two factors: (1) the coherence of the images (perceptually distorted to perceptually intact) and (2) their affective salience (neutral or highly aversive). Our findings provide additional support for the suggestion that the frequency following and frequency doubling SSVEPs have distinct functional roles in the human visual system. The amplitude of the 1F visual evoked potential was modulated in a linear fashion, with

the lowest amplitudes for the most distorted and highest amplitudes for the most intact images, regardless of hedonic content. By contrast 2F SSVEP responses were modulated according to hedonic salience - aversive scenes evoked larger amplitudes compared to neutral scenes, consistent with prior evidence from other imaging modalities. The visual system seems to balance two competing demands - providing high fidelity sensory representations of its environment and selectively enhancing the representation of salient features therein - via temporal segregation of its responses.

Disclosures: **V. Miskovic:** None. **K. Kuntzelman:** None. **N. Hansen:** None. **N. Fletcher:** None.

Poster

088. Emotion: Information Processing

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 88.03/Z19

Topic: F.03. Motivation and Emotion

Support: NSERC

Title: Emotional music and voices: a magnetoencephalography study

Authors: ***K. LOGIE-HAGEN**[1,2], S. RIGOULOT[3,2], J. L. ARMONY[3,2], P. JOLICOEUR[4,2,5]; [1]McGill, Montreal, QC, Canada; [2]Intl. Lab. for Brain, Music, and Sound Res. (BRAMS), Montreal, QC, Canada; [3]Dept. of Psychiatry, Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [4]Dept. of Psychology, Univ. de Montréal, Montreal, QC, Canada; [5]Ctr. de Recherche en Neuropsychologie et Cognition (CERNEC), Montreal, QC, Canada

Abstract: Music is similar to the human voice in its auditory complexity and both can serve different functions, such as convey emotional information. However, the specificities of neural networks involved in the processing of music and voice are currently widely debated. As such, we used magnetoencephalography (MEG) to investigate the similarities and differences in how humans process emotional music and voices. Participants (N=21) listened to human vocalizations, as well as short (1.5 sec) musical excerpts played with saxophone, piano, or violin. Prior to the study the auditory stimuli were rated as sad, happy, fearful or neutral. Source localization (dSPM) revealed significant differences in spatial and temporal activation patterns between vocalizations and music, particularly within the temporal lobes, consistent with previous fMRI results. Specifically, vocalizations elicited stronger activation in both hemispheres during the magnetic equivalent of the P50, compared to music. Vocalizations also resulted in slightly

more temporal/frontal lobe activation in later components (200 ms onwards). In turn, musical excerpts evoked significantly stronger responses during the magnetic equivalent of the N100, especially in the right temporal lobe. These differences between music and human vocalizations were mainly driven by piano. In contrast, violin elicited similar patterns in magnetic signal to those of vocalizations, which is likely due to the similarity of violin to the human voice in their frequency. In terms of emotion, the largest differences were observed between happy and sad stimuli. By using emotionally complex auditory stimuli across various domains, results from this MEG study can provide valuable insights into how music and human voices are processed in the brain. Our study is also useful as a means to assess the strengths and limitations of the MEG approach in cognitive affective research.

Disclosures: K. Logie-Hagen: None. **S. Rigoulot:** None. **J.L. Armony:** None. **P. Jolicoeur:** None.

Poster

088. Emotion: Information Processing

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 88.04/Z20

Topic: F.03. Motivation and Emotion

Support: 2014-15 Psi Chi Undergrad. Res. Grant

Title: Altruism effects on event-related potentials following violent video game play

Authors: *R. D. FARERO[1], J. J. FOWLER[1], J. HOST[1], K. BRALEY[1], C. PHIEL[2], M. A. KISLEY[3], D. S. ALBECK[1];
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Abstract: Previous research has shown that playing violent video games alters a person's behavior and physiology. In the present study electrical signals from the brain were recorded by electroencephalography (EEG) in order to capture the neural response to emotionally charged visual images before and after playing a violent video game. Participants were shown a total of 360 images; with 24 violent, 24 positive and 312 emotionally neutral images, as rated by previous similar studies. Level of altruism was collected through a self-report survey. EEG responses to violent images were isolated, filtered and averaged. Preliminary results indicate that an individual's altruism level is correlated to within subject change in average amplitude of

visually-evoked responses to violent images r= 0.689, n= 13, p<.01. The within subject change was calculated by subtracting individual's average amplitude of visually-evoked responses before playing a violent video game by the average amplitude after playing a violent video game. A greater visually-evoked EEG response implies the brain is allocating more cortical activation to the stimulus, thus a greater level of attention when viewing the images. This increase may suggest that playing the violent video game sensitized the brain of higher altruistic individuals toward violent images. Additionally, the lower altruistic individuals had less cortical activation after playing the video game suggesting desensitization towards the violent images after playing a violent video game. The oxytocin receptor genotype in these participants is also currently being studied.

Disclosures: R.D. Farero: None. **J.J. Fowler:** None. **J. Host:** None. **K. Braley:** None. **C. Phiel:** None. **M.A. Kisley:** None. **D.S. Albeck:** None.

Poster

088. Emotion: Information Processing

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Program#/Poster#: 88.05/Z21

Topic: F.03. Motivation and Emotion

Support: 2014-15 Psi Chi Undergrad. Res. Grant

Title: The magnitude of violent acts committed in a video game alters the EEG response to violent images shown after playing the game

Authors: *D. S. ALBECK[1], J. J. FOWLER[1], C. PHIEL[2], M. A. KISLEY[4], J. HOST[3], K. BRALEY[3], R. FARERO[3];
[1]Dept Psychol, [2]Dept Integrative Biol., [3]Psychology, UC Denver, Denver, CO; [4]Psychology, Univ. of Colorado Colorado Springs, Colorado Springs, CO

Abstract: Emotionally charged images evoke greater electrophysiological responses in the brain than neutral images, as measured by scalp electroencephalography. In this study we are using event-related potential (ERP) methodology to study how playing a violent video game affects the neural response to different types of images. Participants were shown a total of 360 images; 24 violent, 24 positive and 312 emotionally neutral, as rated in previous studies. Preliminary data has found that participants who committed the greatest number of violent acts during 15 minutes of video game play exhibited a significantly stronger ERP than those who committed the fewest number of violent acts in the game. ERP responses were quantified by the average amplitude of

the late positive potential (LPP) elicited by image presentation. Measurements were taken at the PZ zone, with the ERP in for the violent images being statistically significant, low vs. high violence ($t(11) = 3.28$, $p < .05$). Oxytocin receptor genotype in these participants is also currently being studied.

Disclosures: **D.S. Albeck:** None. **J.J. Fowler:** None. **C. Phiel:** None. **M.A. Kisley:** None. **J. Host:** None. **K. Braley:** None. **R. Farero:** None.

Poster

088. Emotion: Information Processing

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 88.06/Z22

Topic: F.03. Motivation and Emotion

Support: UH College of Engineering

Title: Emergent cortical dynamics during aesthetic experiences

Authors: *K. KONTSON[1,2], M. MEGJHANI[2], J. BRANTLEY[2], J. G. CRUZ-GARZA[2], S. NAKAGOME[2], D. ROBLETO[3], M. WHITE[3], E. CIVILLICO[1], J. CONTRERAS-VIDAL[2]; [1]U.S. Food and Drug Admin., Silver Spring, MD; [2]Dept. of Electrical and Computer Engin., Univ. of Houston, Houston, TX; [3]Menil Collection, Houston, TX

Abstract: In this work, the brain response to conceptual art was studied through electroencephalography (EEG) to characterize the neural basis of aesthetic judgments and experiences. To ensure recording of neural dynamics during natural cognition, participants were not constrained to a laboratory environment as in previous studies, but were moving and thinking freely as they viewed an exhibit by artist Dario Robleto at the Menil Collection in Houston, TX. The brain activity of a diverse group of 127 participants was recorded using two dry-electrode mobile EEG headsets (actiCAP Xpress - Brain Products, Germany; StarStim - Neuroelectrics, Spain) and one gel-based wireless, mobile EEG headset (actiCAP - Brain Products, Germany). Data were segmented according to each piece in the exhibit and grouped into three classes describing the complexity of the pieces viewed based on the calculated perceptual features (gradient, luminance, texture, and composite features) of the art pieces. Features in the time, frequency, and wavelet domain were extracted from the EEG data and used as inputs to machine learning tools to classify different patterns of brain activity associated with viewing artworks of varying complexity. The maximum classification accuracy obtained was 55% ± 1.03%, which was determined to be statistically greater than chance level at 33% (paired sample t-test, $p <$

0.01). To understand the neural networks engaged during aesthetic perception and judgment, a functional analysis of dynamic brain activity was performed in participants using the reference gel-based system. Results revealed a significant increase in connection strength between several predefined regions of interest while participants viewed one of the complex pieces of art deemed by participants to be the most aesthetically pleasing compared to viewing a blank wall. Viewing an art piece with complex perceptual features resulted in early recruitment of posterior visual areas followed by focal activations of frontal areas. Differences in the strength of connections while viewing a complex piece of art were also observed for participants of different ages and gender. The results of this work may shed light on how aesthetic experiences engage the brain to stimulate creativity, and could lead to development of innovative neurotechnologies intended to restore or enhance aesthetic reactions in neurologically-impaired persons while allowing to study the brain 'in action and context'. Disclaimer: The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of HHS.

Disclosures: **K. Kontson:** None. **M. Megjhani:** None. **J. Brantley:** None. **J.G. Cruz-Garza:** None. **S. Nakagome:** None. **D. Robleto:** None. **M. White:** None. **E. Civillico:** None. **J. Contreras-Vidal:** None.

Poster

088. Emotion: Information Processing

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 88.07/Z23

Topic: F.03. Motivation and Emotion

Title: Personality traits predict insular activation during anticipation of affective pictures: an fMRI

Authors: ***K. MAKITA**[1], N. KANAYAMA[1], T. UYAMA[1], G. OKADA[1], T. SASAOKA[1], M. MACHIZAWA[1], K. ONODA[2], S. YAMAWAKI[1];
[1]Hiroshima Univ., Hiroshima, Japan; [2]Shimane Univ., Izumo-shi, Japan

Abstract: Anxiety is an important mechanism for generating adaptive behavior as it guides appropriate responses to undesirable events; however, an excessive anxiety becomes problem. Previous findings suggest that the score of "Harm Avoidance (HA)" measured by Temperament and Character Inventory (TCI), an inventory for personality traits, assesses individual's tendencies who has passive avoidance for incoming undesirable events or pessimistic anxiety for one's future. Studies have suggested that a person with high HA score has a risk factor for

development of depression and anxiety disorders (Cloninger et al., 2006). However, neural bases of personality traits pertaining risk factor of depression remains unclear. This study was undertaken to determine the neural locus of brain activity correlated with HA scores during anticipation of pictures which induce emotional feelings. 16 healthy subjects were participated in this study (21~26 years, 8 men). Before scanning in MRI, subjects completed TCI questionnaire, then were subjected to a cued reaction time task in MRI. In the task, a warning tone was presented on each of a series of trials and followed by an emotional picture. The pictures were selected from the International Affective Picture System (IAPS) (Lang et al., 2008). Two types of warning tones (High: 4000Hz, Low: 500Hz) were created. High tone served as a certain cue for a subsequent negative picture (negative anticipation); Low tone was followed by a positive picture (positive anticipation). After the picture presentation, subjects were required to respond their feelings by button press. As a result, subjects' scored 37~61 in HA (mean=50.9, SD=±5.59). These HA scores were entered into the whole brain regression analysis, and we found that subjects' HA scores significantly predicted the degree of activation in a network of brain regions including left insula during anticipation of negative compared to positive pictures. Subjects who have higher tendency of HA showed relatively higher activation in the left insula during negative anticipation compared to positive anticipation. Based on previous study that reports increased insula's activation when one direct one's attention on something unknown (Grinband et al., 2006), our results suggest that a person who has higher anxiety (higher HA scores) tend to respond to incoming unpleasant events more sensitively compared with the others (lower HA scores). In terms of an adaptive behavior, as is important to anticipate and prepare for the events which can happen in the future, a person with higher anxiety are prone to evaluate future negative events excessively, thereby inducing a risk to have depressive mood.

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Poster

088. Emotion: Information Processing

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R21MH100268-02

Title: Increased dorsomedial prefrontal cortex and precuneus activation precede correct emotion identification

Authors: *M. A. ORLOFF, M. C. COFFMAN, A. TRUBANOVA, M. RULOFF, S. W. WHITE, D. GRACANIN, I. KIM, M. A. BELL, S. M. LACONTE, J. A. RICHEY; Virginia Tech., Blacksburg, VA

Abstract: Background: There are six basic emotions that are inborn and recognized across virtually all cultures. Many individuals with mental disorders such as schizophrenia, ADHD, and ASD demonstrate difficulties identifying certain emotions nonverbally, via facial expression. We sought to identify which brain regions are responsible for the emotion misidentification in a healthy adult sample to inform problems that could be occurring in clinical populations. Objectives: Utilize fMRI to characterize brain regions that are differentially activated immediately prior to correct or incorrect emotion identification. Hypothesis: We predicted that the fusiform gyrus (FG) would show decreased activation prior to incorrectly identified faces, as compared to correctly identified faces due to its role in face processing. Methods: Male participants (N=20, mean age=23.4, mean FSIQ=110) were recruited from the community. During fMRI, participants viewed slow-motion videos of faces morphing from neutral to one of the six basic emotions (happiness, sadness, fear, disgust, surprise, and anger). Participants were instructed to indicate once they recognized the displayed emotion and then asked to choose which of the emotions was shown. Each subject completed 30 trials, with an average of five faces displaying each emotion. Preprocessing was accomplished using SPM8. Onset times and durations of events were used to model a signal response containing a regressor for each response type (correct and incorrect), which was convolved with a double-gamma function to model the hemodynamic response of the 2 seconds prior to the button press, indicating recognition. Results: There was increased activation in both the dorsomedial prefrontal cortex (dmPFC) and the precuneus in the two seconds immediately preceding correct identification ($p<0.01$, FDR corrected). Contrary to our initial hypothesis, no significant activity was observed in the FG. Conclusions: Our findings demonstrate that increased activation in the dmPFC and the precuneus immediately precedes accurate, but not inaccurate judgments of other's emotions. Consistent with this finding, Ochsner et al. (2004) found that both the medial prefrontal cortex and the precuneus were active in judgments of both self and others across all basic emotions. Lack of between-group activation differences in the FG suggest that emotion identification accuracy was not due to difficulties with facial processing.

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Poster

088. Emotion: Information Processing

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Program#/Poster#: 88.09/Z25

Topic: F.03. Motivation and Emotion

Title: Beyond eyeballing: modelling event-related pupil responses with canonical response functions

Authors: *C. W. KORN[1], D. R. BACH[1,2];
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Abstract: Pupil responses have recently received renewed interest as they may allow inferring psychological and neural variables of information processing in emotional and motivational processes. Previous studies analyzed baseline-corrected averages, or directly regressed variables of interest on pupil size. Up to now, a unified and principled analysis approach for pupil responses is lacking. This is surprising given the established use of biophysical models for analyzing neuroimaging and psychophysiological data, for example in the framework of general linear convolution models (GLM). Here, we establish such a GLM approach for event-related pupil responses. To derive the biophysical properties of pupil responses, we presented participants (n = 23) with five different luminance levels for 5 s each (120 trials). Dilations and constrictions differed in shape, showing that pupil time series are not simply the output of a linear time invariant (LTI) filter. Still, the system could be approximated by combining two LTI filters. One filter takes the luminance time series as continuous input, incorporating the dilation response and a non-linear relation between luminance and steady-state pupil size. A second filter models the difference between dilation and constriction by taking a brief unit input for each positive luminance change. We derived canonical impulse response functions (CRFs) from our data and analytically approximated them with gamma functions. A GLM based on the two CRFs explained 60.1% of the variance in our data (SD across participants = 13.3). Crucially, this GLM explained more variance than a baseline model which only took steady-state pupil size into account (47.7%, SD = 11.2). The results were robust for different filter settings and CRF approximations. Notably, the model makes the counter-intuitive prediction that constrictions dominate the responses to brief flashes of both light and darkness. This was confirmed in an independent group of participants (n = 14) with luminance changes of 200 ms. Here, our GLM explained 43.6% of the variance (SD = 11.9) while the baseline model explained a negligible variance proportion (0.3%, SD = 0.2). Based on luminance-evoked pupil changes, we have characterized the biophysical properties of event-related pupil responses in a GLM approach. The same biophysics should apply to cognitive influences on pupil size and we will apply the model to cognitive tasks. Analyses routines will be available in the PsPM toolbox (pspm.sourceforge.net). Our parsimonious mathematical description of pupil responses will be

helpful for refining their relation to behavioral and brain indices of emotional and motivational processes.

Disclosures: C.W. Korn: None. **D.R. Bach:** None.

Poster

088. Emotion: Information Processing

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Program#/Poster#: 88.10/Z26

Topic: F.03. Motivation and Emotion

Title: Exploring the origins of learned salience: valuable, risky, novel, and aversive

Authors: W. GRIGGS, A. GHAZIZADEH, *O. HIKOSAKA;
Lab. Sensorimotor Res., Natl. Eye Inst., NIH, Bethesda, MD

Abstract: An object is considered salient if it attracts attention/gaze more strongly compared to its surrounding. While the role of physical features in bottom-up salience has been studied extensively, systematic examination of causal origins of nonphysical salience has been lacking. Here we examined various factors that may change an object's salience through experience (learned salience). Random computer-generated fractals with no prior life experience were used to create object histories from scratch. We monitored eye movements of macaque monkeys (n=4) while they freely viewed objects that differed only by a single experiential factor, namely: value, risk, novelty or aversiveness. Objects were differentially trained on these factors for >5 days prior to testing in free viewing. We measured salience by the ability of the object to attract and maintain gaze during free viewing in the absence of any behavioral outcome. For value contrast, sets of 5 random fractals were associated with increasing amount of juice reward (0%, 25%, 50%, 75%, 100% of max juice). For risk contrast, sets of 5 random fractals had the same mean juice reward but increasing variance around the mean (0%, 25%, 50%, 75%, 100% of max variance). Analysis of gaze in free viewing showed a monotonic increase in attraction and maintenance of gaze as a function of object value. Risk free viewing also caused longer viewing times, but in contrast with value, risky objects did not necessarily attract more saccades. Third, free viewing of novel vs. familiar fractals showed higher attraction and retention of gaze to novel objects, with decreasing effect as the novelty wore off. Finally, objects with aversive but non arousing outcomes (time-out or aversive tastants) showed weaker gaze bias compared to neutral objects. On the other hand, objects associated with an arousing and aversive outcome (airpuff) showed increased gaze bias. Therefore, for aversive objects, arousal or threat seems a better predictor of salience than negative valence. Our results suggest that, while the final outcome for

all these experiential dimensions may look the same (i.e. gaze bias), the underlying mechanisms are diverse. Such multiple mechanisms for learned salience may cooperate or compete to bias attention and gaze.

Disclosures: W. Griggs: None. **A. Ghazizadeh:** None. **O. Hikosaka:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: NIA Grant R01AG038043

Title: Noradrenergic mechanisms of arousal-biased competition in memory

Authors: *D. CLEWETT[1], M. SAKAKI[2], S. NIELSEN[1], G. PETZINGER[1], M. MATHER[1]; [1]USC, Los Angeles, CA; [2]Univ. of Reading, Reading, United Kingdom

Abstract: Selection is at the core of efficient cognitive processing. According to the Arousal-Biased Competition (ABC) model, arousal amplifies the stakes of on-going selection processes, leading to “winner-take-more” and “loser-take-less” effects in perception and memory. Thus, arousal helps optimize memory of important information when it matters most. To explain how this process occurs in the brain, we recently proposed the Glutamate Amplifies Noradrenergic Effects (GANE) model, which posits that arousal-induced norepinephrine (NE) release biases the competition for limited mental and metabolic resources in favor of prioritized (e.g., goal-relevant) information. According to GANE, an activity-dependent increase in local norepinephrine leads to the activation of β-adrenoreceptors and, as a result, the selective enhancement and consolidation of a high priority trace. The goal of this human pharmacological study was to test GANE theory using a double-blind, placebo-controlled, randomized design in which 26 healthy younger adults were administered 40mg of propranolol, a β-adrenoreceptor blocker, or 40mg of vitamin E placebo. After pill administration, participants completed an emotional oddball task in which they were asked to prioritize a neutral object appearing just before an emotional or neutral black-framed image within a sequence of 7 individually presented neutral objects. To assess ABC memory effects, we examined the interactions between arousal, priority and drug condition in corrected recognition memory for oddball-1 (high priority) and oddball+1 (low priority) objects. Task-induced increases in salivary alpha-amylase were also measured to determine how global increases in noradrenergic system activity were associated

with arousal-related modulation of priority effects in memory. Under placebo, emotional relative to neutral oddballs impaired mean recognition memory for lower priority oddball+1 objects but had no effect on memory of high priority oddball-1 objects. This anterograde amnesic effect of arousal was blocked by propranolol. Individuals who recalled more high priority objects preceding an emotional versus neutral oddball image also showed greater task-induced increases in salivary alpha-amylase, indicating that individual differences in NE release induced by the oddball task may be critical for the arousal-induced memory enhancement of high priority oddball-1 objects. Together these findings suggest that norepinephrine mediates the selective influence of emotional arousal on short-term memory.

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Poster

088. Emotion: Information Processing

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 88.12/Z28

Topic: F.03. Motivation and Emotion

Title: Tasteful packaging: How health and ethical messaging can affect the consumer experience

Authors: *M. M. NIEDZIELA, A. JORDAN, H. STONE, M. ROSAZZA;
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Abstract: Ethical buzz words like “organic”, “sustainable”, and “non-GMO” have become increasingly popular for consumer products. Consumer demand for such products is rising rapidly. Health and environmentally conscious consumers are driving sales of products yet it is unknown how much of an impact such claims have on consumer perception. Using a combination of psycho-physiological measures, traditional quantitative questionnaires and conjoint analysis, we aimed to understand the consumer’s experience when exposed to these claims. QUANTITATIVE: Subjects (n=302) were exposed to 12 images of yogurt packaging with varying claims combinations including control (plain package), environmental (“organic”, “sustainable”, “non-GMO”) and dietary (“low fat”, “low calorie”) and were then given a questionnaire to assess perceived taste, brand perception, and cost perception of the stimuli as well as general health and environmental implications on grocery purchases. PSYCHO-PHYSIOLOGICAL: Subjects (n=18) were exposed to 12 images of yogurt packaging with varying claims combinations including a control (plain package), environmental (“organic”, “sustainable”, “non-GMO”) and dietary (“low fat”, “low calorie”) for 8 seconds each while

being measured for HR, GSR, and fEMG. Visual attention was also measured using eye tracking. Subjects were then asked to answer a questionnaire. RESULTS/CONCLUSION: Psycho-physiological data showed that claims helped sustain arousal and engagement with products, but can also have a negative effect on pleasantness and cognitive attention. Environmental and health claims also had an effect on perceived taste and amount consumers were willing to pay for the product with significant differences between males and females and among different age groups. The results of this study provide much needed insight into the importance of consumer package communications for health and environmental issues.

Disclosures: **M.M. Niedziela:** A. Employment/Salary (full or part-time):; HCD Research. **A. Jordan:** A. Employment/Salary (full or part-time):; HCD Research. **H. Stone:** A. Employment/Salary (full or part-time):; HCD Research. **M. Rosazza:** A. Employment/Salary (full or part-time):; HCD Research.

Poster

088. Emotion: Information Processing

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: F.03. Motivation and Emotion

Support: the Academy of Finland (# 259752 and # 273147)

aivoAALTO project

Title: Functional interaction between amygdala and orbitofrontal cortex during the processing of emotionally salient auditory stimuli depends on the emotional valence of the stimuli

Authors: Z. CHEN[1,2], H. LIN[1,2], L. PARKKONEN[2], J. WEI[3], J. DONG[3], Y. MA[3], *S. CARLSON[1,2];
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Abstract: It is well known that amygdala is anatomically connected reciprocally with orbitofrontal cortex (OFC) in human and nonhuman primates (Cavada et al., 2000). The functional connection between these areas is considered to relate to regulation of emotions and impulsive behaviour (Rolls, 2004). Here, we studied whether the strength of this functional connection reflects the processing of the emotional content embedded in naturalistic stimuli. We recorded intracranial local field potentials bilaterally in the amygdala (basolateral nuclei) and

caudal OFC in three Rhesus monkeys while they listened to audio clips (duration 0.95-1.5 s) of recordings of monkey vocalizations that were categorized according to their social context and judgements of human listeners as neutral (coo), threatening (bark), fearful (scream) and happy (warble) monkey calls. We examined the oscillatory synchronization between the amygdala and OFC by using a cluster-based permutation test. The beta-band (15-25 Hz) coherence between the left amygdala and left OFC differed significantly between the monkey calls with different emotional valence; the coherence was stronger to the monkey calls with salient emotional content than to the neutral monkey call, being strongest to the fearful call, and stronger to the threatening and fearful calls than to the happy call. However, the beta coherence between the right amygdala and right OFC in the same conditions was inconsistent in the three monkeys. Our results suggest that the processing of emotionally salient, naturalistic auditory stimuli in the amygdala-OFC axis contributes to the emotion-associated behaviour. References: 1. Cavada C, Compañy T, Tejedor J, Cruz-Rizzolo RJ, Reinoso-Suárez F. Cereb Cortex 2000, 10(3): 220-42. 2. Rolls E. Brain Cogn 2004, 55(1): 11-29.

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Poster

088. Emotion: Information Processing

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 88.14/Z30

Topic: F.03. Motivation and Emotion

Title: Song preference measured by behaviors depends on song similarity in female zebra finch

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Abstract: Male zebra finches (Taeniopygia guttata) court females to sing songs. Male songs have two different types: directed and undirected songs. Directed song is the just directed to the female for courtship, in contrast undirected song is sung alone without female birds. Male's song is different in individual, while females distinguish directed songs, and select the male bird with high preference as her mate. Therefore, male song is one of the important cues for mate-choice and pair bonding in female zebra finches. Females display preference for particular song: conspecific songs over heterospecific songs, familiar songs of the father over unfamiliar conspecific songs, and mate's directed songs over mate's undirected songs. However, how directed song preference of female is related to behaviors is still unknown, and female's song

preference to several directed songs has not been evaluated yet. The purpose of this study is to quantify the directed song preferences of female over several directed songs based on song similarity. Previous studies have estimated the mating preferences of females by choice-chamber experiments. To evaluate the directed song preferences of female in detail, we recorded female behaviors and extracted following six indexes: spatial proximity, hop on perches, tail-quivering or body shakes, production of calls, bill-wipes, and peck keys. These indexes were analyzed by principal component analysis (PCA) in individual subject. Song similarity to mate's directed song is calculated by Sound Analysis Pro (SAP) software. Songs used in this experiment are 15s duration and contain several motifs, so song similarity is estimated as the average of each motif similarity calculated each period of song called motif. The first to third principal components are more than 85% proportion of variance in almost subjects, so three dimensions reduction is appropriate. Euclidean distance to mate's directed song was calculated from the first to third principal components, and we examined the relationship between song similarity and mate's directed song. As a result, song similarity and behaviors show correlation in all females experimented (N=8). This result demonstrates that female tend to perform preference-related behaviors, if song similarity between mate's directed songs and other's directed song is high score. This result indicates that song preference to other's directed song measured by female's behavior depends on song similarity to mate's directed song. There is a possibility to evaluate directed song preference in individual female by behavioral analysis.

Disclosures: **R. Tabata:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

088. Emotion: Information Processing

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 88.15/Z31

Topic: F.03. Motivation and Emotion

Title: The influence of social skill training on the ability of emotional face recognition

Authors: I. B. F. GREBOT[1], *W. C. DE SOUZA[2], A. M. M. NOZIMA[1], J. S. R. AGUIAR[1], G. F. S. FERREIRA[3];
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Abstract: The ability to identify facial expressions of emotion has undisputable evolutionary implications and has been linked to social skills. Similarly, the practice of clinical psychology demands the development of certain social skills, albeit in a specific context. Thus, it could be

expected that individuals within the supervised training stage of the regular psychology undergraduate course would have developed these abilities. The objective of this research was to evaluate possible correlations between the identification of the six basic facial expressions and the undergraduate course stage. This research consisted of a sample of 163 participants, 82.8% female, divided into two groups ("novice" and "veteran"), based on the stage of the undergraduate course. The experimental task consisted on the presentation of a series of facial expression stimuli, divided into static and dynamic. The results show that there was no significant difference in the ability of facial expression identification, between the experimental groups. As suggested by other research in this field, the ability to identify facial expressions demands specific training.

Disclosures: I.B.F. Grebot: None. **W.C. De Souza:** None. **A.M.M. Nozima:** None. **J.S.R. Aguiar:** None. **G.F.S. Ferreira:** None.

Poster

088. Emotion: Information Processing

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Topic: F.03. Motivation and Emotion

Support: Medical Research Council (UK) studentship

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TrygFonden Charitable Foundation

ERC Consolidator Grant CAREGIVING (n.615539)

Title: Motion and emotion: depression reduces psychomotor performance and alters affective movements in caregiving interactions

Authors: *K. S. YOUNG[1,2], C. E. PARSONS[2,3], A. STEIN[2], M. KRINGELBACH[2,3]; [1]UCLA, Los Angeles, CA; [2]Psychiatry, Univ. of Oxford, Oxford, United Kingdom; [3]Aarhus Univ., Aarhus, Denmark

Abstract: Background: Impaired social functioning is a well-established feature of depression. Evidence to date suggests that disrupted processing of emotional cues may constitute part of this impairment. Beyond processing of emotional cues, fluent social interactions require that people physically move in synchronized, contingent ways. Disruptions to physical movements are a

diagnostic feature of depression (psychomotor disturbance) but have not previously been assessed in the context of social functioning. Here we investigated the impact of psychomotor disturbance in depression on physical responsive behavior in both an experimental and observational setting. Methods: In Experiment 1, we examined motor disturbance in depression in response to salient emotional sounds, using a laboratory-based effortful motor task. In Experiment 2, we explored whether psychomotor disturbance was apparent in real-life social interactions. Using mother-infant interactions as a model affective social situation, we compared physical behaviors of mothers with and without postnatal depression (PND). Results: We found impairments in precise, controlled psychomotor performance in adults with depression relative to healthy adults (Experiment 1). Despite this disruption, all adults showed enhanced performance following exposure to highly salient emotional cues (infant cries). Examining real-life interactions, we found differences in physical movements, namely reduced affective touching, in mothers with PND responding to their infants, compared to healthy mothers (Experiment 2). Conclusions: Together, these findings suggest that psychomotor disturbance may be an important feature of depression that can impair social functioning. Future work investigating whether improvements in physical movement in depression could have a positive impact on social interactions would be of much interest.

Disclosures: K.S. Young: None. **C.E. Parsons:** None. **A. Stein:** None. **M. Kringelbach:** None.

Poster

088. Emotion: Information Processing

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 88.17/Z33

Topic: F.03. Motivation and Emotion

Support: James H. Zumberge Faculty Research and Innovation Fund at USC

Title: Conditioned place preference successfully established in typically developing children

Authors: *B. L. THOMPSON[1,2], L. HILLER[2], S. TAKATA[2];
[1]Cell and Neurobio., Univ. of Southern California, Los Angeles, CA; [2]Div. of Occup. Sci. and Occup. Therapy, USC, Los Angeles, CA

Abstract: Affective processing, known to influence attention, motivation and emotional regulation is poorly understood in young children, especially for those with neurodevelopmental disorders characterized by language impairments. New strategies and tools that probe more

complex internal responses, such as feelings, drives, and motivations independent from language, become necessary for populations of children with language delays and other language impairments, and even typically developing children. In this study we faithfully adapted a well-established animal paradigm used for affective processing, conditioned place preference (CPP) for use in typically developing children between the ages of 30-55 months. To our knowledge, this was the first attempt at establishing the use of CPP in children. The current study addressed whether young children could learn a conditioned place preference. The paradigm utilized straightforward Pavlovian conditioning methods in a custom-built child friendly arena to assess whether a preference had been conditioned. Children displayed a robust CPP, with an average 2.4 fold increase in time spent in the preferred room. Importantly, associative learning as assessed with conditioned place preference was not correlated with scores on the Mullen Scales of Early Learning, indicating that CPP can be used with children with a wide range of cognitive skills. Moreover, this technique could also useful for populations with language impairments or delays, as performance in this task does not depend upon expressive language. As such, the utility of this task is seen to reach far beyond the typical pediatric population, and can be a useful probe for understanding motivation and associative learning in children with neurodevelopmental disorders. We note that there are numerous studies employing animal models to study human disorders, but the reverse - adaptation of well-regarded rodent models in studies to further understand human behavior- is the exception. Translational studies like this are necessary for understanding the biological underpinnings of human behavior and disorders. The use of well-conserved, behavioral probes that have clearly defined structure-function relationships in animals provides a unique opportunity to establish similar neurobiological relationships in children.

Disclosures: **B.L. Thompson:** None. **L. Hiller:** None. **S. Takata:** None.

Poster

088. Emotion: Information Processing

Location: Hall A

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Program#/Poster#: 88.18/Z34

Topic: F.03. Motivation and Emotion

Title: Developmental changes in the perception of voluntary and involuntary emotional vocalizations

Authors: *S. H. CHEN, S. KRISHNAN, S. EVANS, S. GULDNER, A. A. GOMES, N. KHAMOSIA, S. SCOTT;
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Abstract: How does age affect the perception of emotion? Laughter and crying are fundamental to the human experience and are produced and heard right from infancy to adulthood. While laughter and crying can be helpless or involuntary, voluntary versions can be deliberately used in social situations. Involuntary laughter is perceived as more contagious than voluntary laughter. Laughter that humans perceive as involuntarily produced elicits greater activity over auditory regions such as the Heschl's gyrus and STG. However, laughter perceived as voluntarily produced elicits greater activity in the anteromedial prefrontal cortex, an area associated with making social inferences (McGettigan et al., 2013). Both temporal and prefrontal regions show protracted changes in grey matter density over development (Gogtay et al., 2004), yet the behavioural consequences of such change on development are unclear. In this study, we investigate the perception of emotional vocalizations across age. In a large-scale study conducted in the Science Museum, London, we tested 1,847 visitors. Participants were played vocalizations varying in voluntariness (involuntary/voluntary) and emotion (laughter/crying); they rated emotional sounds on 5-point Likert scales for their perceived authenticity ('How real do you think the emotion is?') and their contagiousness ('How much do you want to join in?'). Only data from 1,723 participants (1,010 females) who passed our catch-trials criterion was further analysed. The final age-distribution was: 3-9-years, N = 318; 10-17 years, N = 346; 18-23 years, N = 362; 24-29 years, N = 259; 30-59 years, N = 399; 60-76 years, N = 39. Preliminary analyses showed that the developmental trajectories for perception of voluntary and involuntary expressions differed: with age, the difference between authenticity ratings increased (laughter: $R2 = 0.108$, $p < 0.001$; crying: $R2 = 0.161$, $p < 0.001$). The same was true of the contagion ratings (laughter: $R2 = 0.097$, $p < 0.001$; crying: $R2 = 0.053$, $p < 0.001$). Our results suggest a shaping of the response to voluntary sounds over the lifespan, rather than to involuntary sounds. This indicates an important, but differential, role for social factors and experience in learning about the perception of emotional vocalizations.

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Poster

088. Emotion: Information Processing

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: F.03. Motivation and Emotion

Support: Intel Foundation Grant 16237373

National Center for Research Resources 5G12RR003060-26

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NIDA 5 R25 DA035161-03

Title: Compounds found in energy drinks can differentially affect depression in adolescent and adult male rats

Authors: *K. H. CHAUHAN[1], S. L. PEREZ[2], K. URIBE[3], D. WOO[1], U. AKPARA[1], M. EVELYN[1], S. SINGH[1], M. MURITALA[1], S. AYO[1], F. JACQUES[1], S. SOYEMI[1], A. COLE[1], P. DUVALSAINT[1], D. PETERS[3], A. ELZANIE[1], D. HARRIS[1], S. MARACHERIL[1], M. A. GUZMAN[1], A. ALEXANDER –STREET[2], K. Y. SALAS-RAMIREZ[1];
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Abstract: In the United States, nearly 75% of children under the age of 18 consume caffeine, not just in coffee, soda and tea, but also in energy drinks like Red Bull and Monster, which also contain taurine. Energy drinks claim to improve mood, cognition and motor performance. More recently, energy drinks have been shown to decrease the subjective effects of impairment and increase binge drinking. Few animal studies directly compare the behavioral effects of caffeine and taurine in adolescents and adult subjects. The objective of this study was to determine the individual and combined effects of caffeine and taurine on locomotion, anxiety and depression, in adolescent and adult male rats. 32 adolescent and 32 adult male subjects were randomly divided into four groups (1) caffeine (20mg/kg/day; ip), (2) taurine (100mg/kg/day; ip), (3) caffeine & taurine (as a cocktail/day; ip) or (4) saline (ip/day). Treatment was initiated on PND 33 in adolescent males and PND 68 in adult males. One week after treatment began; consecutive behavioral assessments to assess anxiety (elevated plus maze), locomotion (open field) and depression (forced swim) were performed while the males continued to be exposed to these compounds. The energy drink compounds did not affect locomotion or anxiety on either adolescent or adult male rats. However, two-way ANOVAs revealed a main effect of treatment ($p < 0.002$) as well as age ($p < 0.0001$), showing that both adolescent and adult males have reduced immobile time in the forced swim test. Males treated with caffeine and the cocktail containing caffeine and taurine spent significantly less immobile time, while taurine alone had no effects, after three weeks of exposure. These data highlight the antidepressant properties of

caffeine, alone and when it is combined with taurine. In general, adult males spent significantly more immobile time than adolescent males demonstrating that adolescent male display less depressive behavior. Taken together, these data suggest that active compounds found in energy drinks can have differential effects on emotional regulation, dependent on the developmental time of exposure, which in turn can impact behavioral outcomes related to psychopathologies and possibly cognitive function.

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Poster

089. Emotion Processing: Neurophysiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 89.01/Z36

Topic: F.01. Human Cognition and Behavior

Title: Correlational EEG study between love, attachment, and the mirror neuron system

Authors: *E. SHAKOOR, J. E. FISHBEIN, M. PALOMINO, J. A. PINEDA;
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Abstract: When we are in love or attached to our partners, we experience a greater understanding and connection with them; these feelings may be linked to the idea that humans mirror the actions and behaviors of other humans. Hence, topics related to love and attachment have been increasingly explored within this context. One definition of love is the longing to commit to another that emerges from our desire to expand our sense of self to include the person with whom we love. A natural question, then, is whether there is a link between love, attachment, and the human mirror neuron system? We hypothesized that women in a romantic relationship experience increased mirror neuron activity reflected by mu rhythm suppression evident in EEG when they encounter positive and negative stimuli related to their partners as opposed to controls. We studied females in 6 to 18 month long heterosexual romantic relationships and recorded EEG and EKG as they read positive compliments directed to them by their partners, and as they watched videos of their partners receiving a staged slap to the face. The participants' mu rhythm suppressions were compared to those observed when responding to three controls under the same conditions. In order to evaluate and compare the love and

attachment levels of our participants, we administered two surveys_The Love Attitudes Scale, and The Attachment Style and Close Relationships Scale. The former measures six kinds of love: passionate, playful, friendship, practical, intense, and selfless. The latter assesses secure, anxious, and avoidant attachment. Results indicate that love and attachment scales correlate differently with respect to the duration of the romantic relationships. Passionate and friendly love, the two most relevant to the early phases of a relationship, correlate positively with the duration of the relationship, while avoidant and anxious attachment styles correlate negatively. We predict that these participants will show increased mirror neuron activity, as indexed by increased mu suppression when they receive compliments from their partners or when they view aversive stimuli being inflicted on their partners compared to controls. Examining the relationship between love, attachment, and the mirror neuron system in heterosexual couples adds to the known functionality of the mirror neuron system, and furthermore offers a vital link between feelings of romantic love and mu rhythm suppression_one that has yet to be established.

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Poster

089. Emotion Processing: Neurophysiology

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 89.02/Z37

Topic: F.01. Human Cognition and Behavior

Title: Theta oscillations in human approach-avoidance conflict relate to threat memories

Authors: *S. KHEMKA[1,2], G. BARNES[3], R. J. DOLAN[3], D. R. BACH[2];
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Abstract: Simultaneous presence of appealing and aversive aspects of a situation results in approach-avoidance conflict (AAC). Rodent models based on AAC are commonly used to study anxiety-like behaviour (e.g. elevated plus maze, open field, operant conflict). In these test beds, ventral hippocampus but not dorsal hippocampus or amygdala is involved in controlling anxiety-like behaviour. Further, hippocampal local field potential shows increase in theta power and theta frequency compared to familiar environments. A recent functional neuroimaging and lesion study in humans has demonstrated a similar role for the anterior hippocampus, the analogue to the ventral hippocampus in rodents. Using magnetoencephalography (MEG), we have previously also suggested increased hippocampal theta power in human AAC. Here, we seek to relate

human hippocampal theta oscillation specific task aspects and behaviours. We developed a virtual AAC computer game based on rodent operant conflict tests. As an approach incentive, a monetary token was presented in each trial that participants could collect by making a response. Presence of a sleeping "predator" with three different wake up probability served as an avoidance motivation. The predator could wake up and catch the participant, leading to loss of tokens. Theta power from the hippocampus was estimated using linearly constrained minimum variance beamformers. Extending previous findings, our results suggests that induced theta activity (1-4 Hz)in medial temporal lobe is higher during high threat compared to low threat in medial temporal lobe within 1 sec of token appearance, i. e. during the actual conflict. Before the token appeared, i. e. during presentation of the predator context alone, no difference between threat levels was observed. Also, potential loss did not impact on theta power. Further analyses suggest that theta power does not relate to expected outcome or to specific behaviours. We also observe that theta activity after token appearance at low threat level is lower than a pre-trial baseline. To summarize, our results suggest that theta power increases with (learned) threat level but not with the explicitly signaled threat magnitude. This could suggest that it is related to retrieval of fear memories during conflict.

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Poster

089. Emotion Processing: Neurophysiology

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Program#/Poster#: 89.03/Z38

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI Grant No. 26350990 by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Insular lesion and attenuated sensitivity to the emotions of others

Authors: ***Y. TERASAWA**[1], Y. KUROSAKI[3], Y. IBATA[4], Y. MORIGUCHI[5], S. UMEDA[2]; [1]Psychology, Keio Univ., Kanagawa, Japan; [2]Dept. of Psychology, Keio Univ., Tokyo, Japan; [3]Dept. of Communication Disorders, Hlth. Sci. Univ. of Hokkaido, Sapporo, Japan; [4]Nasu Red Cross Hospital,, Ohtawara, Japan; [5]Dept. of Psychophysiology, Natl. Ctr. of Neurol. and Psychiatry, Tokyo, Japan

Abstract: The insular cortex has been considered to be the neural base of visceral sensation for many years. Previous studies in psychology and cognitive neuroscience have accumulated

evidence indicating that interoception is an essential factor in the subjective feeling of emotion. Recent neuroimaging studies have demonstrated that anterior insular cortex activation is associated with accessing interoceptive information and underpinning the subjective experience of emotional state. Only a small number of studies have focused on the influence of insular damage on emotion processing and interoceptive awareness. Moreover, disparate hypotheses have been proposed for the alteration of emotion processing by insular lesions. Some studies show that insular lesions yield an inability for understanding and representing disgust exclusively, but other studies suggest that such lesions modulate arousal and valence judgments for both positive and negative emotions. In this study, we examined the alteration in emotion recognition in three right insular-damaged cases with well-preserved higher cognitive function. Participants performed an experimental task using morphed photos that ranged between neutral and emotional facial expressions (i.e., anger, sadness, disgust, and happiness). Recognition rates of particular emotions were calculated to measure emotional sensitivity. Also, they performed heartbeat perception task for measuring interoceptive accuracy. Their interoceptive accuracy was remarkably low and the cases identified emotions that have high arousal level (e.g., anger) as less aroused emotions (e.g., sadness). The current results show that insular lesions lead to attenuated emotional sensitivity across emotions, rather than category-specific impairments such as to disgust. Our findings support the hypothesis that the insular cortex modulates recognition of emotional saliency and mediates interoceptive and emotional awareness.

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Poster

089. Emotion Processing: Neurophysiology

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Program#/Poster#: 89.04/Z39

Topic: F.01. Human Cognition and Behavior

Support: Grant in Aid for Scientific Research

Title: Electrophysiological correlates of subliminal affective face priming

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Abstract: Purpose: Behaviorally, “affective priming effect” refers to the phenomenon that presentation of affective prime stimuli shifts subsequent affective evaluation to the target stimuli. However, neural correlates of “subliminal” affective face priming effects are largely unknown. Here, we studied neural activities accompanied with subliminal affective face priming effect using high-density ERPs. Methods: Nine healthy volunteers (6 males, mean age 24.1±2.0 y.o.) seated in front of a 20 inch monitor in a silent darken room. Stimulus sequences consisted of two types of 17 ms-subliminal prime faces (i.e. fearful or scrambled faces) followed by a backward mask (SOA 300 ms) and three types of 800 ms-target faces (i.e. neutral, emotionally ambiguous, or fearful faces). Prime and target face stimuli were presented in a random order with 864 trials (144 trials × 2 prime faces × 3 target faces). Subjects were instructed to judge the expression of target faces as neutral or fearful by button-pressing. Response time (RT) and emotional judgment for target faces were recorded. Continuous EEG was recorded by a 128 channels EEG system while subjects performed the task. ERPs were obtained by averaging the response for each target face separately. According to previous reports, N170 and N250r (“r” means “repetition”) in the occipito-temporal area were analyzed. Latency and peak amplitude for each component of ERP was compared by a three-way repeated measures ANOVA (2 types of prime stimuli × 3 types of target stimuli × hemispheres). Results: All subjects accomplished the experiment. Behaviorally, there were no significant differences in RT and emotional judgment for both priming conditions. Regarding ERPs, ANOVA revealed that N170 to the ambiguous target face followed by fearful prime faces was significantly larger than that of scrambled prime faces. N250r to ambiguous target face followed by fearful prime faces was significantly smaller than that of the scrambled prime faces. Conclusions: Although there have been some reports that the N250r is getting larger for repetitions of the same image or familiar face, the N250r to ambiguous faces was reduced by subliminal fearful face priming in this study. This finding is consistent with the phenomenon that the cortical activity for primed stimuli was smaller than that for unprimed stimuli (i.e. “repetition suppression”). Thus, it is concluded that N250r may be sensitive to subliminal affective priming.

Disclosures: **M. Tanaka:** None. **T. Maekawa:** None. **K. Ogata:** None. **N. Takamiya:** None. **E. Yamada:** None. **S. Tobimatsu:** None.

Poster

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Title: Decoding of emotional states during watching movies using multimodal neurophysiological signals

Authors: ***J. CHOI**, J. CHOI, K. KIM;
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Abstract: Emotional status is reflected in the physiological signals on central and autonomic nervous systems (CNS and ANS). The purpose of this study is to investigate whether different emotional states can be decoded from multimodal neurophysiological signals. In particular, we tried to decode a subject's emotional response to affective movies from single-trial electroencephalograms (EEGs) and several signals reflecting the behavior of ANS. Fifteen healthy university students participated in the experiment. Thirty-two affective movie clips reflecting four types of different emotional states were selected from based on the valence-arousal model (high/low valence and high/low arousal). Right after watching every single movie clip, the subjects rated their emotional response to the movie in valence, arousal, dominance, and like/dislike levels. Multichannel EEGs, skin temperature, Galvanic skin response (GSR), and photoplethysmogram (PPG) were recorded during the tasks. The spectral, temporal and spatial characteristics of EEGs showing significant differences among different emotional states were determined to construct feature vectors for pattern classification. The time and frequency domain features of the physiological signals were selected so that they reflect the characteristics of the ANS, and the measures showing significant differences among the emotional states were selected as additional feature vectors. A support vector machine (SVM) with Gaussian radial basis function (RBF) kernel was adopted as a pattern classifier along with principal component analysis (PCA) for dimensionality reduction. The classification accuracy was calculated by ten-fold cross-validation. When only the EEGs were used, the classification accuracies were as high as as 96.58% and 98.07, for the decoding of valence and arousal levels, respectively. It was still as accurate as 90.62 and 90.02% for the case of using only the ANS physiological signals. By using multimodal decoding strategy, i.e., using both the CNS and ANS signals, the classification accuracies were 97.29% and 98.47% for valence and arousal, respectively. Our results demonstrate that the different emotional states to the affective movies can be decoded with high accuracy from multimodal neurophysiological signals.

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Poster

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Support: Innovation Fund of Shanghai Designated for Undergraduate Students

Title: Neural responses to emotion: an event-related potentials study

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Abstract: Human emotion can be elicited by static visual stimuli (e.g. pictures), meanwhile emotional movie as stimulus is emerging in the psychophysiological study of emotion. The present study aims to explore brain activity through electroencephalogram (EEG) recording, during which different affective movies are presented to subjects. Subjects (12 male, 20-21 years old) performed a passive viewing task, using 49 non-auditory affective movie clips from the Emotional Movie Database (EMDB) as stimulus. Film clips of EMDB were estimated to elicit five emotional states (horror, erotic, social negative, social positive and neutral) in the affective space. The amplitude and the latency of event-related potentials (ERPs) component for each emotion were calculated. For the early components of ERPs, P1, N1 and P2 were located at 100 ms, 120 ms and 180 ms, respectively, under each emotional state. The N1 amplitude was stimulus-locked. For the slow components of ERPs, the P300 wave was detected at parietal and occipital lobes. In addition, erotic emotion stimuli elicited the maximal amplitude of P300. One robust negative slow wave, the N450 component, was found in the frontal lobe during 250-450ms after movie onset, and the amplitude of it was emotion-sensitive. The motivated brain area following different emotional stimulus was identified using low-resolution electromagnetic tomography (LORETA). The present results show that different amplitude of stimulus-locked components found in ERPs, such as N1, P300 and N450, may be related to the distinct pattern of neural activity and neural circuit of each emotion stimulus.

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Poster

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Support: JSPS KAKENHI 26120722 (SU)

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Title: Time course and localization of brain activity in humor comprehension: an ERP/sLORETA study

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Abstract: Humor comprehension is a complex process that requires the detection and resolution of the incongruity, eliciting a positive feeling of mirth or reward. Although a number of imaging studies have investigated the incongruity-detection and resolution process, it is difficult to functionally and anatomically dissociate these processes. In this study, we conducted the event-related potentials (ERP) experiment using the same materials in our previous fMRI study (Shibata et al., 2014). We also conducted source localization analysis using standardized low resolution brain electromagnetic tomography analysis (sLORETA) to examine the time course and localization of brain activity in incongruity detection and resolution stages (Pascual-Marqui, 2002). Eighteen participants were instructed to read funny or unfunny scenarios and to judge whether the target sentence is funny or not. Results showed that within the P200 time window, critical words in the funny condition elicited larger P200 amplitudes than those in the unfunny condition in the central electrode sites. Within the P600 time window, critical words in the funny condition elicited larger P600 amplitudes than those in the unfunny condition in the central and parietal electrode sites. The sLORETA analysis on the P200 ERPs revealed a stronger activation for funny condition relative to unfunny condition in the superior frontal gyrus (SFG) and medial prefrontal cortex (mPFC). The sLORETA analysis on the P600 ERPs revealed a stronger activation for funny condition relative to unfunny condition in the middle temporal gyrus (MTG) and inferior parietal lobule (IPL). These ERP results were almost consistent with those of our fMRI study. Taken together, the results of sLORETA analysis and connectivity analysis in our previous fMRI study suggest that the incongruity - detection process activates the SFG and mPFC during the 200-250 ms post-stimulus onset interval, whereas incongruity-resolution process generates activation at the right MTG and IPL during the 580-620 ms post-stimulus onset interval. These results also suggest that the comprehension of humor and literal language already differed during earlier phases of processing, and followed by a P600 component reflecting incongruity-resolution process.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: MH097320

Title: Network interactions during affective picture processing: An ECOG study

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Abstract: Human EEG and fMRI studies have shown that motivationally salient stimuli activate a distributed brain network. What remains not well-understood is how elements of the network interact with one another during affective processing. FMRI's poor temporal resolution and EEG's inability to access deep brain structures limit their utility to address this question. In this study we recorded electrocorticogram (ECOG) from patients undergoing evaluation for surgical therapy to treat intractable epilepsy. The subjects were asked to passively view 20 pleasant (erotica, romantic courtship, sport scenes), 20 neutral/calm (household scenes, people), and 20 unpleasant (mutilation, human violence, attacking animals) stimuli from the International Affective Picture System (IAPS) in random order. Each picture was presented for 1000ms with inter-trial intervals varied from 6000 to 9000ms. A time-frequency analysis of high gamma activity revealed a time period of interest (300 ms to 600 ms). Granger causality was then evaluated in this time period between electrodes in the medial temporal lobe (MTL) and in the posterior lateral temporal lobe (PLTL). It was found that (1) inter-areal communication is bidirectional, (2) it takes place mainly in the theta band, and (3) the strength of interaction, especially MTL->PLTL, is modulated by picture valence. Results were corroborated by analyses of cross-area phase-amplitude-coupling between theta and high gamma. Together, these findings provide initial evidence that emotional perception relies on a wide-spread network of structures communicating through distinct patterns of oscillatory activity.

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Support: 5T34GM008395-25

Title: N170 Differences on emotion recognition between deaf/ hard of hearing and normally hearing individuals

Authors: *C. C. MORALES[1], A. V. GONZALEZ[2], E. M. DUBON[2], J. P. ABARA[2], S.-M. KANG[2];
[2]Psychology, [1]California State Univ. Northridge, Los Angeles, CA

Abstract: The N170 event-related component is associated with early-face sensitivity (Botzel et al.,1995). A study investigating facial processing found that both deaf and non-deaf individuals showed a faster N170 latency with misaligned faces than with aligned faces (Mitchell, Letourneau, & Maslin, 2013). Another study conducted by Utama et al. (2009) found that N170 amplitude was related to intensity levels of facial emotion among healthy subjects. Thus in order to further investigate the relationship between N170 and emotion recognition, this study explored differences in N170 amplitudes of emotion recognition between normally hearing participants and deaf/ hard of hearing individuals. Participants were given an emotion recognition task that contained a whole-face and half-face condition. Participants were instructed to identify the emotions presented on a computer screen, while the even-related brain potentials on the four locations of the head (FZ, CZ, PZ, and Oz) were recorded. A total of 42 images of happy, anger, disgust, surprised, sad, and neutral emotions from the NimStim Face Stimulus Set (Tottenham et al., 2002) were presented for each condition. A mixed ANOVA found a main significant difference between condition, $F(1,13) = 17.93$, $p < .001$, with half-face condition (M=-3.74, SD=1.77) having a higher N170 amplitude than the whole-face condition (M=-1.74, SD=1.86) . At PZ for the half face condition there was a higher N170 amplitude for the deaf group (M=-5.24, SD=1.94) than in the control group (M=-3.24, SD=3.03). At PZ for the whole face condition, there was a higher N170 amplitude for the control group (M=-2.57, SD=2.63) than in the deaf group (M=-.46, SD=.52). These results indicate that the deaf group allocated more neuronal resources in the half-face condition, while this pattern is reversed in the whole-face condition. There was a significant interaction for condition and group for N170 latency $F(1,13) = 6.45$, $p = .025$, with whole-face condition for control (M=136.52, SD=22.72) having a higher N170 latency than the whole face condition for deaf (M=-127.17, SD=27.83) . There was a

higher N170 latency for the half-face condition for the deaf group (M=151.06, SD=19.66) than in the control group in the half-face condition (M=129.78 SD=16.02). These results indicate that for the deaf group the N170 was elicited faster when recognizing emotions when viewing all facial features, whereas the control group had a faster N170 latency for the half-face condition.

Disclosures: C.C. Morales: None. **A.V. Gonzalez:** None. **E.M. Dubon:** None. **J.P. Abara:** None. **S. Kang:** None.

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Title: Self-other differences in mu event-related desynchronization correlate with self-reported perspective-taking

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Abstract: Simulation theories offer an account of empathic perspective-taking by assuming that one understands another's feelings by simulating the other's emotions. While simulation offers an efficient means of adopting the perspective of another, it also poses a problem to be solved. If the emotions one feels can come both from one's own emotional state as well as the emotional state of others, how does the brain determine which emotions belong to the self and which to the other? Building on previous studies using blocked designs, the current study utilized event-related desynchronization (ERD) to analyze the time course of electroencephalographic (EEG) mu rhythm desynchronization and to determine whether correlations between blocked mu suppression and perspective-taking replicate with ERD and perspective-taking. Participants viewed videos clips of their own and others' faces forming one of four emotional expressions. ERD's were computed for 1.5s epochs following stimulus onset. Initial analyses revealed significant self-other differences in ERD between 340-960ms for central and frontal electrodes ($p < .05$ for C4, Fc4, and F4), replicating blocked mu suppression data. Furthermore, a negative association was found between self-reported perspective-taking ($r = -.341$) and mu ERD in electrode FC3 and a positive association was found between perspective-taking and self-other ($r = .371$) ERD difference scores. The self-other ERD differences as well as their correlations with

perspective-taking replicate previous research and extend it by revealing the time course of these differences and correlations.

Disclosures: **T. Depaola:** None. **D.J. Barbera:** None. **C. Woodruff:** None.

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Title: Effects of parasympathetic activation on neural responses during emotional reactivity and regulation in adolescents

Authors: D. G. GHAHREMANI[1], D. VRANEK[1], A. HOLOVATYK[1], A. DEAN[1], *E. D. LONDON[2];
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Abstract: Parasympathetic tone is an important contributor to homeostatic balance and likely has significant influence on the flexibility of cortico-limbic networks involved in regulation of emotion and arousal. Adolescence is a neurodevelopmental period in which cortico-limbic circuitry has not yet reached maturation, likely resulting in greater difficulty with regulation of emotions. We sought to determine how a brief intervention for increasing parasympathetic tone via controlled breathing impacts adolescent emotion reactivity and regulation. Twenty-one adolescents (13-18 years old) underwent two fMRI scanning sessions in which resting state scans were acquired before and after they performed a reappraisal-based emotion regulation task, which involved proximal/distal perspective taking while viewing images containing neutral or negatively arousing content (IAPS pictures). Prior to each scan session, participants engaged in either a controlled breathing exercise known to increase parasympathetic activation or a control condition in which they were guided through a relaxation exercise that involved normal breathing. Parasympathetic tone was measured using heart rate variability during rest in supine position before and after breathing or relaxation. Controlled breathing increased heart rate variability (an indication of greater parasympathetic tone), reduced fMRI activation to emotional images in occipital and posterior parietal areas, and increased activation in dorsolateral prefrontal and striatal regions. These preliminary results suggest that enhancement of parasympathetic

activation results in greater fronto-striatal recruitment during emotional arousal. Given the role of fronto-striatal networks in self-regulation, activation in such networks may reflect greater capacity for self-regulation resulting from increased parasympathetic tone.

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Poster

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Topic: F.01. Human Cognition and Behavior

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Title: Measuring engagement in movie trailers using behavioral and neural responses

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Abstract: A movie trailer is a commercial advertisement that aims at attracting audiences and encouraging them to see the movie afterward. So the movie industry strives to create a better movie trailer that delivers key information and mood of a movie. However, methods to evaluate the effectiveness of trailers have been little changed from a traditional way such as self-reports. Self-report has been criticized in terms of its credibility since participants has limitations on describing their implicit mental states objectively. Neurocinematics has emerged to probe affective and cognitive states of audiences by analyzing neural responses to movie. However, in most neurocinematics studies, self-report was still used to validate the credibility of neurophysiological results. In this neurocinematics study, we addressed this issue by employing a psychophysical task to assess neural responses without relying on subjective reports. We adopted a dual-task framework where participants watched movie trailers as a primary task while they also had to react promptly to a tactile stimulus as a secondary task. We measured a secondary task reaction time (STRT) and brain activity to assess how deeply participants were engaged in trailers. We hypothesized that the more participants were engaged in trailers, the slower STRT would be. A neural engagement index was developed as temporal changes in differences between frontal alpha (7~10Hz) and beta (12~14 Hz) power values of electroencephalography (EEG). After watching each movie trailer, participants reported their

anticipation, fun, attentiveness and preferential rank scores. With the three different measurements, - STRT, neural engagement index and self-reports - we evaluated eight different movie trailers from various genres. As a result, we found a significant correlation between STRT and neural engagement index. On the other hand, there was no significant correlation between self-reports and STRT, or self-reports and neural engagement index. These results may suggest a novel way of evaluating neural data for neurocinematics, highlighting that the credibility of neural engagement index should be assessed using a more objective method instead of using traditional self-reports.

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Topic: F.01. Human Cognition and Behavior

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Title: The parietal cortex and the fearful face recognition

Authors: ***K. ZHAO**[1,2], W. ZHOU[3], Y. LI[4], T. YU[5], L. WANG[2,6];
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Abstract: Fearful faces would convey signals of potential threat and recognizing such facial expressions with precision in conspecifics is evolutionarily advantageous. The predominant view in this field was that the amygdala plays a significant role in the recognition of such faces. In contrast, some studies did not duplicate this finding, which raises a possibility that the achievement of this function may originally come from other cortical areas projecting to the amygdala. Lesion studies have demonstrated that the damage of the parietal region, particularly to the right side, can affect the processing of fearful faces. However, it remains unclear in human how neural activities in the parietal cortex respond to such faces. Neural oscillations,

prominently evident in the local field potentials (LFPs) recorded in many brain areas, serve as a potentially temporal reference. In this study, we employed a modified Brief Affect Recognition Test paradigm. During this task, a flashing face from one of four categories (fearful, surprising, happy or neutral) occurred after a fixed fixation period and epileptic patients with implanted electrodes were instructed to report its category. Intracranial recordings (ECoG or SEEG) were simultaneously performed during the task running. The behavioral results showed that both fearful and surprising expression were easily misrecognized as seen in our previous studies. Meanwhile, clear-cut results showed a significantly increased high-gamma (80-200 Hz) power in the inferior parietal area and the supramarginal gyrus only for fearful faces before a judgment. This specificity was not found for surprise, happy, or neural face identification. In contrast, the recording from the amygdala did not show such categorizing function. These findings suggest that the parietal cortex, especially in the supramarginal gyrus other than the amygdala, play vital roles in recognizing fearful face.

Disclosures: **K. Zhao:** None. **W. Zhou:** None. **Y. Li:** None. **T. Yu:** None. **L. Wang:** None.

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Topic: F.01. Human Cognition and Behavior

Title: Contralateral attention-related attenuation of occipitotemporal electrocortical activity by backward masked snakes & guns

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Abstract: Signals of environmental threat capture observers' attention. Fearful facial expressions enhance the amplitude of the face sensitive N170 event-related potential at occipitotemporal electrode sites in the hemisphere contralateral to their presentation. However, non-face visual threats, such as snakes and guns, also capture spatial attention; although it is unclear how visual cortical processing is affected by these threat types. To address this knowledge gap we ran a dot-probe task to measure spatial attention elicited by backward masked threat stimuli including snakes, spiders, knives, and guns while reaction time and electroencephalogram data were collected (N = 14). Electroencephalogram data were segmented and time-locked to the onset of the backward masked threat stimulus. The behavioral results

suggest that backward masked threats capture spatial attention, F (1, 13) = 11.18, p = 0.005. Reaction times were faster for threat-congruent (M = 320.15 ms) compared to threat-incongruent (M = 325.84 ms) targets. The event-related potential data indicated a hemisphere × visual field interaction on the N1 at occipitotemporal electrode sites (F (1, 11) = 9.80, p = 0.01) where amplitudes where attenuated in the hemisphere contralateral to the threat stimulus. Thus, non-face threats capture attention by attenuating early contralateral occipitotemporal electrocortical activity.

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Topic: F.03. Motivation and Emotion

Title: Heart rate variability in dogs: Toward a valid behavioral model of empathy in companion animals

Authors: *J. MANOR, M. BOTTEN, W. CHU, E. P. WIERTELAK;
Psychology, Macalester Col., Saint Paul, MN

Abstract: Domestic dogs have a unique and close relationship with humans. Dogs seem to show sensitivity and empathy towards the emotional states of humans (e.g., Custance & Mayer, 2012), but it has been difficult to distinguish empathetic behavior from curiosity or seeking self-comfort. This study aimed to measure a physiological marker of empathy in domestic dogs through heart rate variability (HRV) monitoring and behavior analysis. Heart rate variability (HRV) is the beat-to-beat variation in heart rate (HR). HRV represents a measure of autonomic nervous system functioning and inhibitory control. In children, HRV measures have been used to link high autonomic regulation with more positive and intense emotionality as well as higher ratings of sympathy toward others (Eisenberg et al., 1996). In the present study, testing was done by recording HRV and behavioral responses in 16 dogs in four experimental conditions: owner is crying, owner is laughing, stranger is crying, and stranger is laughing. The results indicate that there was no significant difference between the owner and stranger in any condition, but that crying elicits significantly more person-oriented/empathetic behaviors than laughing or talking. There was also a significant negative correlation between the number of person-oriented behaviors exhibited and HRV in the stranger crying condition. Interestingly, only for dogs with high baseline HRV, there was also a strong negative correlation between HRV and person

oriented behaviors during the owner crying condition. This physiological change in HRV that occurs with increased behavioral response to a distressed individual suggests that dogs are experiencing the distress of the individual and responding in an empathetic manner.

Disclosures: J. Manor: None. **M. Botten:** None. **W. Chu:** None. **E.P. Wiertelak:** None.

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Topic: F.01. Human Cognition and Behavior

Support: Sleep Research Society Foundation

Title: Changes in autonomic arousal elicited by human amygdala stimulation are parameter-dependent

Authors: *J. T. WILLIE[1], C. S. INMAN[1], D. I. BASS[1], R. E. GROSS[1], S. HAMANN[2]; [1]Neurosurg., Emory Univ. Sch. of Med., Atlanta, GA; [2]Psychology, Emory Univ., Atlanta, GA

Abstract: The amygdala, located within the medial temporal lobe, regulates emotional responses, motivation, and memory. However, few contemporary studies have used direct electrical stimulation of the amygdala in humans to examine stimulation-elicited physiological and emotional changes, and the nature of such effects remains unclear. To determine the effects of amygdala stimulation on acute autonomic physiology, we utilized epilepsy patients undergoing intracranial EEG monitoring in which depth electrodes were implanted surgically from a lateral temporal approach into unilateral or bilateral amygdala. Subjects underwent either sham or acute monopolar electrical stimulation at various parameters in electrode contacts located in either amygdala or within the lateral temporal cortex. Stimulation was applied at either 50 Hz (pulse width of 300 μsec) or 130 Hz (pulse width of 90 μsec), while amplitudes were increased from lower (4 ≤ mV) to higher (>4 mV, 12 mV maximum) amplitudes in a stepwise fashion, with subjects blinded to stimulation condition. Varying pulse widths for each frequency were chosen to balance the charge density delivered across frequencies. Skin conductance responses (SCR), respiratory rate, heart rate, and electromyographic Hoffman-reflex amplitudes, and video images were recorded. At stimulation amplitudes well below patients' subjective awareness of stimulation, and without eliciting any seizures, we found that increasing linear, dose-responsive stimulation effects, with higher-amplitude amygdala stimulation (but not lateral control or sham stimulation) eliciting rapid and significant heart rate deceleration and increasing

skin-conductance. This pattern of results parallels stimulation findings with animals and is consistent with orienting/defensive physiological responses observed with aversive visual stimuli. Notably, all the described physiological effects were observed below thresholds in which patients reported being conscious of stimulation or changes in mood. Such changes occurred only at higher stimulation amplitudes. In a subsequent experiment, ongoing emotional responses to emotional videos were also not interrupted by amygdala stimulation. More intense stimulation may be required to elicit subjective emotional responses such as fear that have been reported previously. In summary, these findings suggest that acute amygdala stimulation in humans is safe and can reliably elicit changes in emotion physiology without significantly affecting subjective emotional experience providing a useful paradigm for investigation of amygdala-mediated modulatory effects.

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Topic: F.03. Motivation and Emotion

Support: R01 MH094489

Title: The effects of RMTg lesions on the response of nigral dopamine neurons to footshock and habenula stimulation: an electrophysiological study in anesthetized rats

Authors: *P. D. SHEPARD, H. L. PALACOROLLA, P. L. BROWN, D. B. BRADY, R. P. MCMAHON, G. I. ELMER;
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Abstract: GABA neurons in the rostromedial tegmental area (RMTg) are an important source of inhibitory input to dopamine (DA) neurons within the substantia nigra (SN). Dense innervation of the RMTg by glutamatergic efferents from the lateral habenula (LHb) provide the hodological substrate for a feedforward inhibitory pathway that underlies the transient inhibition in DA cell firing elicited by aversive stimuli ranging from the loss of an expected reward to peripheral nociceptive stimulation. Here, we sought to determine (i) the degree of confluence in the response of individual SN DA neurons to footshock and habenular activation and (ii) whether excitotoxic lesions of the RMTg altered this response pattern. Extracellular single unit recordings

were obtained from DA neurons in the SN of sham (n= 40 cells) or RMTg lesioned rats (n= 34 cells). 7-14 days prior to the recordings, rats received a midline injection (110 nl) of ibotenic acid (400 mM) or saline. The number of NeuN and amphetamine-induced cFos positive neurons in RMTg lesioned rats was reduced by 87% and 92%, respectively. Spontaneous activity was recorded for several minutes prior to applying brief biphasic constant current pulses pseudorandomly at 0.5 Hz to the ipsilateral LHb (1 mA, 100 usec) or contralateral footpad (5-7 mA, 1 ms). Neuronal responses were categorized as: inhibition (I), excitation (E), inhibition followed by excitation (I/E), excitation followed by inhibition (E/I) or no change (N/C). The majority of cells recorded in sham-treated rats showed a biphasic I/E response to LHb stimulation (36/40). The response to footshock was more heterogeneous but comprised predominantly of cells exhibiting either an I/E (n = 14) or I (n=19) response. RMTg-lesioned rats showed significantly greater variability in their response to LHb stimulation with 50% of the cells characterized by an initial excitation (n=6), NC (n=9) or an indeterminate response (n=2). The increased heterogeneity was accompanied by a disproportionate reduction in the number of I/E cells in lesioned animals (11/34). Comparable changes were observed in the response to footshock including a significant reduction in the proportion of cells characterized by an I/E response (6/34) and a corresponding increase in the NC category. Notably the proportion of cells exhibiting a monotonic inhibitory response to footshock was not altered by RMTg lesions (14 vs12). These data suggest that activation of the RMTg by aversive stimulation has a biphasic effect on SN DA neurons that reflects both the direct and indirect influence of the RMTg.

Disclosures: P.D. Shepard: None. **H.L. Palacorolla:** None. **P.L. Brown:** None. **D.B. Brady:** None. **R.P. McMahon:** None. **G.I. Elmer:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 90.01/AA9

Topic: F.04. Neuroethology

Title: Spatial working memory compared in pigeons and humans

Authors: J. E. WOLF[1], J. F. MAGNOTTI[2], J. O. TAYLOR[1], R. LEE[1], *K. J. LEISING[1]; [1]Dept. of Psychology, 298920, Texas Christian Univ., Fort Worth, TX; [2]Baylor Col. of Med., Houston, TX

Abstract: Change detection procedures are commonly used to assess the capacity (number of objects) of working memory. Previous comparisons of working memory between human and

non-human animals have focused on object-based ("what") working memory, but this is only one aspect of memory. In our study, we trained pigeons and humans to complete a spatial ("where") change detection task. In this task, subjects selected (peck/touch) the item that changed location across a brief delay. Both pigeons and humans performed above chance at selecting the one changed item among the 2, 3, or 4 items in the sample and test displays, as well as across delays of 0, 100, or 1000 ms. From these data we computed spatial working memory capacities for number of locations. We also tested both species with trials in which a visual mask was inserted into the delay following the sample display. The data from pigeons and humans indicate that the mask was most disruptive on trials with a short delay (e.g., 100 ms), and on trials with greater numbers of items (e.g., 3 and 4). These results are the first to compare spatial working memory in pigeons and humans in a change detection task, and indicate the use of iconic memory at delays of 100 ms or less and working memory at longer delays in both species.

Disclosures: **J.E. Wolf:** None. **J.F. Magnotti:** None. **J.O. Taylor:** None. **R. Lee:** None. **K.J. Leising:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

Location: Hall A

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Program#/Poster#: 90.02/AA10

Topic: F.04. Neuroethology

Title: A comparison of c-fos expression in avian hippocampus after relational task and associative task

Authors: *O. F. LAZAREVA[1], M. J. ACERBO[2], M. STACHO[3], O. GÜNTÜRKÜN[3], A. GERTSBERG[1], J. RICK[1], K. PANFIL[1], J. FEUCHT[1];
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Abstract: Recently, Lazareva, Kandray, and Acerbo (2015) showed that hippocampal lesion in pigeons selectively impairs relational behavior in a transitive inference task. Here, we have explored the involvement of hippocampus in another traditional relational task, transposition. Unlike transitive inference task, transposition task does not require manipulating relational information; instead, animals are simply trained to learn the physical relation between the stimuli (e.g., larger or smaller) and use the same relation when presented with novel pairs of stimuli. We trained pigeons to discriminate two pairs of circles of different sizes, S1-S2 and S3-S4 (where numbers indicate a constant increase in circle size from S1 to S4). Some pigeons were trained to

always select a smaller (or a larger) circle in each pair (relational group). The other pigeons were trained to select a smaller circle in one pair and a larger circle in another pair (associative group); in other words, these pigeons had to memorize each stimulus instead of relying on their relationship. After all birds has reached criterion, they were tested with two novel pairs, S2-S8 and S2-S3. Behavioral results indicated that relational group indeed learned to always select a smaller (or a larger) circle, while associative group learned to select previously reinforced circle and avoid previously nonreinforced circle. Next, we analyzed c-fos expression in four hippocampal areas: dorsomedial ventral (DMv) area similar to mammalian CA1; dorsalmedial dorsal (DMd) area similar to CA3; and, dorsolateral ventral (DLd) and dorsolateral dorsal (DLd) areas similar to entorhinal cortex. We found significantly higher levels of c-fos expression in relational group than in associative group for all four areas, although the difference was most pronounced in DLv. These results suggest that hippocampus may be involved in relational tasks that are based on a physical relationship and that do not explicitly require encoding or manipulating relationships between different training stimuli.

Disclosures: **O.F. Lazareva:** None. **M.J. Acerbo:** None. **M. Stacho:** None. **O. Güntürkün:** None. **A. Gertsberg:** None. **J. Rick:** None. **K. Panfil:** None. **J. Feucht:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 90.03/AA11

Topic: F.04. Neuroethology

Title: Blockade of GABA and glutamate receptors in nucleus subpretectalis/interstitio-pretecto-subpretectalis impairs figure-ground discrimination in pigeons

Authors: *M. J. ACERBO[1], O. F. LAZAREVA[2], S. ATTERBERG[2], H. MOSES[2];
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Abstract: Figure-ground segregation is a fundamental visual ability that allows an organism to separate an object from its background. Our earlier research has shown that nucleus rotundus (Rt), a thalamic nucleus processing visual information in pigeons, together with its inhibitory complex, nucleus subpretectalis/interstitio-pretecto-subpretectalis (SP/IPS), is critically involved in figure-ground discrimination (Acerbo et al., 2012; Scully et al., 2014). Here, we further investigated the role of SP/IPS by conducting bilateral micro-injections of GABAergic receptor antagonist and agonist (bicuculline and muscimol, respectively) and glutamate receptor antagonist (CNQX) after the pigeons mastered figure-ground discrimination task. We used two

doses of each drug (bicuculline: 0.1mM and 0.05mM; muscimol: 4.4mM and 8.8mM; CNQX: 2.15mM and 4.6mM), and alternated drug injections with baseline (ACSF) to eliminate potential carryover effects. The order of injections was randomized across birds, and figure trials and background trials were analyzed separately for each drug and dose. We found that all drugs produced a dose-dependent impairment in figure-ground performance. Low doses of bicuculline and CNQX impaired figure accuracy but had no effect on background accuracy; in contrast, high doses of both drugs impaired background accuracy while recovering figure accuracy to near-baseline levels. Finally, muscimol produced an equivalent, dose-dependent impairment on both figure and background trials. Together, these results further confirm our earlier hypothesis that inhibitory projections from SP/IPS to Rt modulate figure-ground discrimination, and suggest that figure-ground discrimination requires a precise balance between excitatory and inhibitory inputs.

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Poster

090. Sensory and Motor Systems in Vertebrates

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

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Topic: F.04. Neuroethology

Support: NIH training grant 5T32HD007094-37

Title: Mechanosensory specialization of duck trigeminal ganglia neurons occurs before hatching

Authors: *E. R. SCHNEIDER[1], E. O. ANDERSON[1], M. MASTROTTO[1,2], W. LAURSEN[1,2], E. O. GRACHEVA[1,2], S. N. BAGRIANTSEV[1];
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Abstract: Organisms evolve varied prey detection strategies depending on ecological niche, and these adaptations can provide a rich source of information for sensory biology. For instance, tactile-foraging waterfowl depend on their acutely touch-sensitive bill to feed in murky water. The glabrous skin covering the bills of these birds is densely populated with mechanoreceptors tuned to detect transient touch and vibration (Berkhoudt et al, 1980, Netherlands J. Zoology 30:1). We have shown that dissociated neurons of trigeminal ganglia (TG) innervating the bill in tactile-foraging waterfowl are optimized to detect mechanical stimuli, with a majority of neurons expressing the mechanotransducer Piezo2 in tactile-specialized vs. non-specialized species. In

the domestic duck, the majority of TG neurons have fine-tuned their ability to detect mechanical stimuli by lowering response threshold and increasing gain (Schneider et al, 2014, PNAS, 111:14941). During development, neurons from duck TG show increased sensitivity to mechanical stimuli as early as four days prior to hatching, compared to embryonic TG neurons from chicken, a bird without tactile specialization, at a comparable developmental stage (duck current at 6 μm stimulation = 2.3 ± 0.4 nA, mean ± s.e.m., n=40; chicken, 0.6 ± 0.2 nA, n = 18, $p < 0.005$, Kruskal-Wallis test). The majority of embryonic duck TG neurons responded to mechanical stimulation (66.7%) whereas only 17.8% of chicken neurons were mechanosensitive (n = 60 duck; 102 chicken). This is consistent with our histological observations that the majority embryonic ducks contain Piezo2 mRNA (69.3%) compared to only 34.9% of embryonic chicken TG neurons. We have previously shown the adult duck TG has significantly fewer neurons containing mRNA for thermosensors TRPV1 and TRPM8 compared to duck dorsal root ganglia (DRG), and TG of non-specialized species. Prevalence of these markers in embryonic duck TG did not significantly differ from adult TG. These observations suggest that the specialization of duck embryonic TG neurons to mechanosensation occurs prior to hatching, consistent with ducks precocial ability to forage soon after hatching, and dependence on the sense of touch for foraging behavior.

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Poster

090. Sensory and Motor Systems in Vertebrates

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Topic: F.04. Neuroethology

Support: KAKEN24120521

KAKEN25290009

KAKEN25650120

Title: Pretecto-hypothalamic circuit is essential for prey perception and feeding behavior in zebrafish

Authors: *A. MUTO, K. KAWAKAMI;
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Abstract: One of the important tasks for vision is to discriminate objects into categories such as food and non-food based on their visual features. In lower vertebrates, the initial step of visual recognition of possible prey, or a small object, starts in the retina. The major target of the retinal projections, the optic tectum of the midbrain has a role in computation of object size and also serves as visuotopic map to locate the prey. Although one component of the premotor circuits for prey capture is shown to be reticulospinal neurons in the nucleus of the medial longitudinal fascicle (nMLF), it is not known whether visual information about the prey directly goes to only this premotor pathway to exert prey catching behavior, or there is another pathways in which visual information converges to an dedicated neuronal module, or a small subset of prey detecting neurons during visuomotor integration for feeding control. Here we show that genetic identification of pretecto-hypothalamic circuit that is essential in prey detection in zebrafish larvae. Subpopulation of the pretectal neurons were activated in the presence of prey. Laser-ablation of these pretectal neurons specifically abolished prey capture behavior. Furthermore, pretectal neurons projected to the inferior lobe of the hypothalamus (ILH), the feeding center in fish, which also showed neuronal activity correlated to the pretectal activity in the presence of prey. Together, these results suggest that the pretecto-hypothalamic circuit serves as a prey detector at the interface of the sensory and motor systems and possibly convert visual perception of food to feeding behavior in zebrafish. The neuronal module dedicated to cognition of prey may provide a place for modification of feeding behavior by internal states and past feeding experience.

Disclosures: A. Muto: None. **K. Kawakami:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

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Topic: F.04. Neuroethology

Support: NSF Grant CMMI-0941674

Title: Oscillatory movement correlates with sensory noise in active electrosense

Authors: *C. CHEN[1], I. D. NEVELN[1], M. A. MACIVER[2];
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Abstract: Spatiotemporal dynamics of different sensory systems often demand specific motor repertoire, which is referred as active sensing. A particular type of weakly electric fish (E.

virescens), will oscillate its body back and forth (a.k.a. whole-body oscillation) to gather information when tracking a movable object using electrosense. However, when using vision, this extra oscillatory movement disappears. From an information theoretic perspective, whole-body oscillation can be considered as a behavioral response to help compensate for the lower contrast and weak signals associated with electrosense. This is confirmed by experimentally showing that the whole-body oscillation amplitude increases with lower signal-to-noise ratio (SNR), which is achieved by electronically jamming the fish (shown to deteriorate electrosense) during controlled tracking experiments. Therefore, it is reasonable to believe that the whole-body oscillation might belong to a specific motor repertoire which is controlled by electrosensory feedback such that its amplitude varies with different ratios of signal to noise within electrosense. To better understand and quantify this and other similar active sensing motor patterns, we have been applying control and information theory. We simulated a mobile sensor tracking a linearly moving object with varying levels of noise. Using a control strategy that distributes sensory measurements proportionate to the expected information density, the resulting sensor trajectory has similarities to whole body movement patterns of the fish using electrosense. The simulated sensor moves in an oscillatory manner alongside the object while also tracking the object movements. Moreover, this pattern diminishes after increasing the SNR in simulation, which resembles fish's behavior when using vision - a sensory modality that has less measurement noise in clear water and daylight conditions.

Disclosures: **C. Chen:** None. **I.D. Neveln:** None. **M.A. MacIver:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

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Topic: F.04. Neuroethology

Support: JSPS KAKENHI Grant 26330278

Title: Non-periodic responses to sinusoidal electric stimulation in ampullary electroreceptors of glass catfish

Authors: *Y. ADACHI[1], K. TATENO[2];
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Abstract: One-dimensional return map analysis was applied to reveal nonlinear response to sinusoidal electric stimulation in ampullary electroreceptors of transparent glass catfish (Kryptopterus bicirrhis/minor). External electric stimulation modulates spontaneous afferent nerve impulses via ampullary electroreceptors. The electroreceptors are sensitive to the sinusoidal electric stimulation around 10 Hz. One-dimensional return maps were constructed from afferent nerve impulses. Glass catfish (5-7cm long) were placed on an experimental chamber, which filled with aerated water of 23 °C ± 2. The fish were anesthetized by 125mg/l of MS-222. The stimulation electrodes were placed in front of and behind the fish. The sinusoidal electric current was passed between a pair of the stimulation electrodes. Stimulation frequency was 1, 2, 4, 8, 10, 20, 40, 80, and 100 Hz. The glass microelectrode was set in an ampullary canal. Spontaneous nerve impulses showed regular beating with little fluctuation. In the one-dimensional return map, all points clustered around a fixed point. Interpulse intervals of nerve impulses were modulated by the sinusoidal electric stimulation in the frequency range between 2 and 40 Hz. Interpulse intervals of the nerve impulses were shorten by the depolarization and prolonged by the hyperpolarization. The one-dimensional return map of the nerve impulses were classified into 2 types: four segments arrayed to an L shape or a triangular-shaped ring. The L shape maps were caused by a burst of nerve impulses. Although the burst of nerve impulses were induced repeatedly, interpulse intervals in the burst of nerve impulses were not exactly the same as the previous intervals. Those one-dimensional return maps did not form a cluster at a fixed point, but four line segments. For the triangular return map, the density of intervals of nerve impulses moderately varied. The triangular map did not have a stable fixed point, and interpulse intervals were non-periodic. The latency of first impulses in each stimulation phase were also collected. The latency of first impulses were not fixed. Those results indicate that the sinusoidal electric stimulation induced non-periodic responses of the nerve impulses. The frequency range of the non-periodic responses is consistent with those of avoidance behavior of glass catfish. We conclude that non-periodic responses of electroreceptors may trigger the avoidance behavior of glass catfish.

Disclosures: **Y. Adachi:** None. **K. Tateno:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

Location: Hall A

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Program#/Poster#: 90.08/AA16

Topic: F.04. Neuroethology

Support: FQRNT

Title: The role of SK channels in optimizing neural processing and behavioural perception of natural stimuli in the electronsensory system

Authors: *C. HUANG[1], M. J. CHACRON[1,2];
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Abstract: The understanding of how neural circuits perform key computations underlies the fundamental knowledge of how cellular machinery leads to neural network activity, which in turn determines perception/behavioral responses. Several studies have shown that neural coding strategies in many sensory systems are adapted to the natural scene statistics to efficiently encode sensory stimuli as well as to maximize redundancy reduction in spatiotemporal correlations. Despite this, the molecular and/or neural network mechanisms underlying such optimal adaptation are poorly understood in general. Here we investigated how small conductance calcium-activated potassium (SK) channels contribute to optimizing neural coding and perception of natural second-order envelopes, which are found ubiquitously across sensory systems and carry important spatiotemporal information necessary for perception. In order to do so, we used the electrosensory system of the gymnotiform weakly-electric fish *Apteronotus leptorhynchus* as a model for its unique advantages for *in vivo* awake-behaving experiments. We recorded from sensory pyramidal neurons in the electrosensory lateral line lobe (ELL) in response to naturalistic envelope stimuli and demonstrated that these neurons are optimally tuned to encode information over a continuous range of envelope frequencies. To better understand this adaptation process, we built a simple phenomenological model that included SK channels. Computational simulations of this model predicted that the strength of SK currents were essential in determining whether tuning was optimal to the natural scene statistics. We tested our modelling predictions by both pharmacologically inactivating and over-activating SK channels in pyramidal neurons and demonstrated that the changes in levels of SK channel activation led to predictable changes in neural tuning and behavioural perception to natural stimuli. Our results therefore show for the first time a novel function of SK channels in that they provide a cellular mechanism which gives rise to optimal neural responses and behavioural perception to natural stimuli in a vertebrate model system. The strong homology between SK channels and the weakly-electric fish to their mammalian counterparts suggest that it is very likely that our results will be generally applicable across systems and species.

Disclosures: C. Huang: None. **M.J. Chacron:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

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Topic: F.04. Neuroethology

Support: NSF EFRI-BSBA-0938007

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Title: An optimal neural encoding model predicts anti-correlated spike-trains in the p-type afferents of a weakly electric fish

Authors: E. C. JOHNSON, D. L. JONES, *R. RATNAM;
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Abstract: P-type afferents of weakly electric fish are the primary sensory neurons of the electrosensory system. Objects in the immediate environment of the fish modulate the self-generated Electric Organ Discharge (EOD) and are encoded as modulations in the instantaneous firing rate of P-type afferents. The spike trains of these afferents exhibit strongly anti-correlated interspike intervals (ISIs) which are presumed to facilitate optimal detection of weak signals generated by small prey. From a computational perspective, spike-timing models with dynamic thresholds have been shown to generate spike-trains with strong anti-correlations. Recently, we proposed a deterministic, optimal, energy-constrained neural encoder, with a dynamic threshold that functions as an built-in decoder. We show that the dynamic threshold (decoder) reconstructs the EOD modulations from the spike-train. The reconstruction is compared with the EOD modulations to feed back an internal copy of the coding error to the encoder (spike generator). The encoder generates spike-trains with optimally timed spikes so as to minimize decoding error subject to a constraint on the rate at which spikes are fired. Here, we extend the deterministic model to stochastic spike-trains. A stochastic spike-firing rule is introduced to create jitter in the predicted spike-times. The optimally encoded spike-times were compared with experimentally determined spike-times from P-type afferents. As with the deterministic optimal coder, the spike-times for the stochastic encoder and the P-type afferent were in close agreement with one another. Of interest here is that spike-trains from the optimal coder demonstrate ISI correlations and ISI return-plots which are very close to the experimental data. Further, anti-correlations are known to stabilize firing-rate (or spike-count) over multiple time-scales. Thus, maintaining a stable firing rate is a natural outcome of the energy (spike-rate) constraint implicitly built into the optimal coder. We conclude that anti-correlated ISIs are a signature for optimal encoding in the electrosensory system.

Disclosures: **E.C. Johnson:** None. **D.L. Jones:** None. **R. Ratnam:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

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Topic: F.04. Neuroethology

Support: Sloan Research Fellowship

NIDCD 5T32 DC008553

Title: Dopaminergic modulation of pre-pulse inhibition in larval zebrafish

Authors: *J. P. BARRIOS[1], S. ANJEWIERDEN[2], J. NEWTON[2], S. LUKS-MORGAN[1], R. DHINGRA[1], A. D. DOUGLASS[1];
[1]Neurobio. and Anat., [2]Bioengineering, Univ. of Utah, Salt Lake City, UT

Abstract: Prepulse inhibition of the startle response (PPI) has been the primary experimental measure of sensorimotor gating in both clinical patients and animal models for decades. This simple reflex modulation is known to be deficient in patients with a range of psychiatric disorders including schizophrenia, obsessive-compulsive disorder and Tourette's syndrome. Treatment of clinical patients and work in animal models has revealed that this behavior is dependent on the neuromodulator dopamine (DA). PPI and PPI deficits are intriguing clinical observations and they raise equally intriguing basic questions about the nature of fast reflex modulation by DA. Interestingly, this behavior is conserved in zebrafish and present in larval fish as early as 5 days post fertilization. We have shown that audiomotor PPI can be evoked and monitored in 6-8 day old zebrafish in a head-fixed context. Subthreshold prepulses given 200, 400 and 800ms before the startling pulse prevented c-bend startle responses in over 30% of trials. The percentage of trials showing inhibition of startle behavior was positively correlated with inter-stimulus interval such that the 800ms delay produced significantly more startle inhibition than the 200ms delay. Interestingly, trials that lack the stereotypical c-bend often show a distinct motor behavior characterized by a long latency to initiation, low angular velocity and low total tail bend angle. These observations constitute the basis for studying the inhibition of the startle reflex by DA in a whole-brain context, taking advantage of the transparency and small size of the larval zebrafish brain. This head-fixed preparation allows us to use whole-brain two-photon calcium imaging and spatially targeted optogenetics to study the circuit mechanisms driving this fast modulation of the acoustic startle reflex. We are currently using this preparation to identify the DA neurons responsible for PPI in these animals focusing in particular on a population of TH2-expressing cells in the hypothalamus. We will use whole-brain calcium imaging to

determine how DA transforms the c-bend startle behavior into a routine swim. These experiments will provide a comprehensive map of the circuits that underlie PPI and allow us to define the mechanisms by which endogenous dopaminergic neurons shape the behavior.

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Poster

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Topic: F.04. Neuroethology

Support: FCT PTDC/AAG-REC/2488/2012 (NEUTOXMER)

Title: Neurotoxicity of inorganic mercury in white seabream (Diplodus sargus) - morphofunctional brain alterations and behavioural shifts following waterborne exposure and post-exposure periods

Authors: S. PUGA[1], P. PEREIRA[1,2,3], F. PINTO-RIBEIRO[1], J. RAIMUNDO[3], O. ARAÚJO[3], M. PACHECO[2], *A. A. ALMEIDA[4];
[1]Life and Hlth. Sci. Res. Inst. (ICVS), Sch. of Hlth. Sciences, Univ. of Minho, Campus Gualtar, 4710-057; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Braga, Portugal; [2]Dept. of Biol. and CESAM, Univ. of Aveiro, 3810-193, Aveiro, Portugal; [3]IPMA - Portuguese Inst. for the Sea and Atmosphere, Av. Brasília, 1449-006, Lisbon, Portugal; [4]Univ. Minho, Braga, Portugal

Abstract: The metal mercury (Hg) is extremely neurotoxic to humans and wildlife. In aquatic environments, Hg is present in organic (mainly methylmercury - MeHg) and inorganic (iHg) forms. Both Hg species can be bioaccumulated and biomagnified in fish, inducing toxic effects. In fact, high levels of Hg were recorded in fish brain, along with neurodegenerative damage, disturbances on sensory processing, and behavioural changes. Nevertheless, it remains to be clarified which Hg species (iHg versus MeHg) is mainly accumulated in the fish's brain, as well as the contribution of different uptake routes (water versus diet). In this study, we evaluated the swimming performance of the white seabream (D. sargus), and identified alterations in brain morphology together with Hg accumulation in brain and eyes. Initially, fishes were exposed to realistic levels of iHg in water (2 µg L-1) during 7 (E7) and 14 days (E14). After that, fish were allowed to recover for 28 days (PE28). At E7, exposed fish exhibited a significant decrease of

the first swimming distance, as well as a lower time for refuge. In parallel, exposed fish also presented lower total cell number in the optic tectum, cerebellum and hippocampus, together with high levels of Hg in brain and eyes. At PE28, previously exposed fish still swam a smaller distance in the first run, exhibited a lower resistance against the water flow (measured as time to immobility), and presented lower total cell number in the optic tectum. Accordingly, Hg levels in eyes and brain did not decrease during the recovery period. Realistic levels of waterborne iHg can alter fish swimming performance, and trigger total cell number loss in several brain areas responsible for endocrine regulation, sensory and motor-related systems, fine movement and equilibrium/posture without a rapid reversibility. Such impairments could have repercussions in the organism's fitness and survival, leading to increased vulnerability to predation with significant implications for food chain transfer of Hg.

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Poster

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Topic: F.04. Neuroethology

Support: NSERC Post Doctoral Fellowship to HF

CIHR grant to LM

Title: Stimulus-evoked serotonin dynamics in a teleost sensory system

Authors: *H. FOTOWAT[1], E. HARVEY-GIRARD[1], R. CACHOPE[2], J. F. CHEER[3], R. KRAHE[4], L. MALER[1];
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Abstract: We used weakly electric fish, Apteronotus leptorhynchus, to study the temporal dynamics of serotonin release in a primary sensory area. These fish use a self-generated quasi-sinusoidal electric field for communicating with conspecifics. The electric organ discharge frequency (EODf) is individual- and sex-specific and thus the presence of a conspecific causes a net electric field with amplitude modulations at the difference frequency (Df). Electroreceptors

on the fish's skin respond to these modulations and project to pyramidal neurons in the electrosensory lateral line lobe (ELL). The ELL is comprised of four distinct topographic segments, with the lateral segment (LS) specialized for processing electrosensory inputs that arise during interactions with conspecifics. Pyramidal neurons within this segment receive a robust serotonergic innervation and the postsynaptic consequence of serotonin signaling is to increase their bursting probability. The natural time-course of serotonin release in the context of agonistic encounters, however, is unknown. To address this gap in our knowledge, we used Fast-Scan Cyclic Voltammetry to measure, with a temporal accuracy of 0.1 seconds, the dynamics of serotonin release in the ELL-LS. Sensory stimuli consisted of brief encounters with same- or opposite-sex conspecifics simulated via application of a weak electric field at various Dfs to the fish tank. Such stimuli evoked a serotonin-like signal in the ELL-LS. Remarkably, these stimuli were significantly more likely to evoke a serotonin-like response in male than in female fish. Males were also more likely to exhibit a behavioral response (fast increases in their EODf; chirps) in the context of agonistic encounters. Spontaneous serotonin-like release events occurred in some of the trials without electrosensory stimulation. The probability of spontaneous release was, however, significantly lower than release in trials with stimulation. The relation between the probability of release and the identity of the simulated conspecific, i.e. the Df value, was highly variable across, but consistent within individuals. This result underlines the impact of an individual fish's past experience on the probability of release. Interestingly, loud auditory stimuli failed to evoke a response in the ELL-LS suggesting sensory modality specificity. Our results provide previously unseen insight into the time-course of stimulus-evoked dynamics of serotonin-like signals (ongoing experiments are aimed at pharmacological verification of the serotonergic nature of the signals) in a primary sensory area and its possible involvement in shaping the responses of sensory neurons.

Disclosures: H. Fotowat: None. **E. Harvey-Girard:** None. **R. Cachope:** None. **J.F. Cheer:** None. **R. Krahe:** None. **L. Maler:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 90.13/AA21

Topic: F.04. Neuroethology

Title: Social isolation alters socially induced serotonergic fluctuations in the inferior colliculus

Authors: *S. M. KEESOM, L. M. HURLEY;
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Abstract: Appropriate behavior during a social encounter requires that an animal be attuned to the details of social context, and past social experience plays an important role in shaping sensory responses to social signals. Mechanisms that mediate the influences of social experience on sensory systems have been less explored. In the auditory system, serotonin is strategically situated for mediating effects of social experience on sensory processing for three reasons: 1) the serotonergic system itself is sensitive to social experience; 2) socially triggered serotonergic fluctuations reflect behavioral differences among social encounters; and 3) serotonin alters evoked responses of auditory neurons to natural vocalizations. In this study, we directly tested how past social experience affects the acute social serotonergic response in the inferior colliculus, a region involved in processing vocalizations. We used carbon fiber voltammetry to measure serotonin during social encounters of male mice (Mus musculus) that were previously group-housed or individually housed for four weeks. When paired with an intruder male, both group-housed and individually housed mice demonstrated similar elevations in serotonin. However, social isolation altered the trajectory of serotonergic responses over multiple social encounters in a subset of mice from which we obtained repeated measurements. In this subset, group-housed mice had increased serotonergic responses during a second social encounter, whereas individually housed mice had drastically diminished serotonergic responses during the second encounter. Social isolation also altered the relationship between the serotonergic response and social behavior. For group-housed mice, serotonin was positively related to social investigation. In contrast, there was no relationship between serotonin and social investigation for individually housed mice. Thus, social housing influences both the serotonergic system's response to social context over multiple encounters and how it reflects variations among encounters of the same context. These results suggest that prolonged social isolation disrupts the ability of the serotonergic system to convey information about immediate social context to an auditory region.

Disclosures: S.M. Keesom: None. **L.M. Hurley:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 90.14/AA22

Topic: F.04. Neuroethology

Support: UCLA Acad. Senate

Title: Seeing with sound - auditory-visual information transfer in Egyptian fruit bats

Authors: *W. METZNER[1], C. SCHILLING[2], A. GRINNELL[1];
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Abstract: Echolocating bats are able to use echoes of emitted sounds as a substitute for vision. They can catch small flying insects at night with as much facility as a swallow or flycatcher can during the daytime. Their skill at using sound for orientation and food capture is considered one of the most spectacular adaptations of any sensory system, and has spawned a very active field of research. These bats act as if they can form a three-dimensional acoustic image of the world with the echoes returning from one sound emission equivalent to what one would see with a single strobe illumination. It is extremely difficult to imagine how they do this, or indeed to prove that they can. Is the mental image they get from echoes anything like the visual image one gets with vision, or is it an entirely acoustic experience that does not get translated into the kind of gestalt that we and other visually orienting animals are familiar with. We propose to ask this question first of bats that have good vision, but are still capable of accurate echolocation. This is the case for an old world fruit bat, Rousettus, which lives in caves or tombs and uses echolocation when flying in the dark, but also has good vision and uses vision when there is enough light. Specifically, we asked two questions: 1. Can Egyptian fruit bats acquire a mental image of an object using vision (or hearing) alone? 2. Once such a mental image has been formed, can it be transferred from one sensory modality (e.g., vision) to another (e.g., hearing - or the other way around)? Using positive conditioning, we trained the bats to first localize a rewarded object (an X) that was presented pseudorandomly at either the left or right side using either vision (group 1; 3 bats) or hearing (group 2; 3 bats) alone. After only a few weeks, all experimental bats were able to perform these tasks far above the 75% level. Subsequently, we used a forced-choice alternative training paradigm to have the bats discriminate between a rewarded object (the X) and an unrewarded object (a cross) using either vision (group 1) or hearing (group 2) alone. Again, all bats performed above the 75%-level after only a few weeks of training. Finally, we tested if the same bats could also discriminate the objects using the other sensory modality, i.e. hearing in group 1 and vision in group 2. We found that only after a few sessions (much sooner than in the initial discrimination tasks, i.e., vision in group 1 and hearing in group 2), the bats performed this task correctly. This suggests that the bats can indeed, and amazingly easily, transform a mental image that they obtained using one sensory modality (vision in group 1 and hearing in group 2) to another modality.

Disclosures: W. Metzner: None. **C. Schilling:** None. **A. Grinnell:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

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Program#/Poster#: 90.15/AA23

Topic: F.04. Neuroethology

Support: NSF Grant IOS1010193

Title: Neural activity in the SC of a flying echolocating bat

Authors: ***N. B. KOTHARI**, M. J. WOHLGEMUTH, C. F. MOSS;
Johns Hopkins Univ., Baltimore, MD

Abstract: The superior colliculus (SC) is a laminated midbrain structure implicated in species-specific orienting behaviors. Previous recordings from the SC of head restrained and passively listening bats revealed two classes of cells: 1) neurons that encode 2D head-centric space (azimuth and elevation) around the bat and 2) 3D neurons that encode not only the azimuth and elevation but also range. Range is encoded by neurons that exhibit pulse-echo delay tuning. SC recordings from bats resting on a platform and tracking a moving target revealed vocal-premotor activity prior to each sonar vocalization. Here we present extracellular recording data from the SC of freely flying bats, and characterize sensory, sensori-motor and motor neural activity. We trained big brown bats to locate and land on a platform for a food reward. While the bat performed this task, single unit activity was recorded across the SC laminae using a telemetry system. Synchronized with neural recordings, high-speed audio and video recordings captured the bat's echolocation, head aim and flight behaviors. To reconstruct the timing of the echoes arriving at the bat's ears, we developed an echo model using the recorded sonar vocalizations, the bat's 3D head aim and position in space. Correlating echo timing information with neural activity we characterize neurons that respond to echoes from different objects along the bats flight trajectory. Correlating the neural activity with the sonar vocalizations we find sensory-motor and motor neurons in the intermediate and deeper layers of the bat SC. Recording simultaneous activity across the SC laminae allows us to observe the integration of information across these functional layers. Such integration of information is essential for spatial navigation, orientation and prey capture by echolocating bats.

Disclosures: N.B. Kothari: None. **M.J. Wohlgemuth:** None. **C.F. Moss:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 90.16/AA24

Topic: F.04. Neuroethology

Support: EPSRC Doctoral Prize Fellowship

Title: On holey ground: vibrissal touch aids the placement of safe footing during locomotion

Authors: *K. ARKLEY[1], B. T. O'CONNOR[1], R. A. GRANT[2], B. MITCHINSON[1], T. J. PRESCOTT[1];
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Abstract: The earliest mammals were small, nocturnal creatures, capable of highly varied and adaptable motor behaviors. It can be argued that the first mammals, or their therapsid reptilian ancestors, evolved a specialization of the outer epidermal layers of the integument – hair – with a predominantly tactile function. As a result of the sensory advantages of these tactile hairs, the early mammals were able to occupy a variety of new habitats, evolving a more sophisticated motor repertoire than their reptilian ancestors and the insulating pelagic hair. It is known that the *vibrissae* (whiskers) play a significant role in the guidance of fast-paced locomotion by providing a 'look-ahead' to detect upcoming obstacles [Arkley et al., *Curr Bio*, 24(13), 1507-12], but here, we test the hypothesis that whisker movements are also employed by the rat to guide the placement of safe footfalls. Using high-speed videography, whisker tracking and a pedobarograph, we compare foot placement, and whisker and head movements of functionally-blind rats locomoting on both a flat and holey floor from top-down and side-on views, obtaining kinematics in both the rostrocaudal and dorsoventral dimensions. As previously found, animals locomoting along a flat floor adopted a pronounced 'look-ahead' strategy at higher locomotion speeds, with more exploratory whisking movements at slow travel. When travelling along a holey floor, rats successfully avoided placing their feet in holes 95% of the time, appearing to modify their vibrissal and locomotor behaviours in order to monitor the area directly in front of the snout, whilst sampling the contours of the ground in order to guide safe footfalls. This was achieved by maintaining a head parallel with the floor, allowing consistent whisker contact with the ground, and by adopting frequent periodic whisker movements. The position of the whiskers about the snout were also much more bilaterally asymmetric, suggesting the position of the whiskers are constantly updated to monitor the location of holes, analogous to rats exploring an object. Analysis of whisker and head kinematics from a side-on view confirms that some whiskers make direct contact with the floor at all times during locomotion on the holey floor, but deviate during fast-paced locomotion on the flat floor. It seems likely that the differences in whisking and locomotion on a holey and flat floor arise as a product of the same strategic goal – safe locomotion. In particular, our data suggest vibrissal input during locomotion on uneven

ground informs the animal of the most appropriate place to position the feet, as evidenced by the overlapping spatial area of the floor that both snout and forepaws cover.

Disclosures: **K. Arkley:** None. **B.T. O'Connor:** None. **R.A. Grant:** None. **B. Mitchinson:** None. **T.J. Prescott:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

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Support: NIH grant P30 HD18655

NSF Graduate Research Fellowship

Stuart H.Q. & Victoria Quan Fellowship

Human Frontiers Science Program

Harvard/MIT Joint Grants program fellowship

NSF IIS-1421780

Burroughs Wellcome Fund

Title: Sub-second structure in mouse behavior

Authors: *A. B. WILTSCHKO[1,3], M. J. JOHNSON[3,2], G. IURILLI[2], R. E. PETERSON[2], J. M. KATON[2], S. L. PASHKOVSKI[2], V. M. ABRAIRA[2], R. P. ADAMS[3], S. R. DATTA[2]; [2]Dept. of Neurobio., [1]Harvard Med. Sch., Boston, MA; [3]Sch. of Engin. and Applied Sci., Harvard Univ., Cambridge, MA

Abstract: Complex animal behaviors are believed to be built out of simpler modules, which can be flexibly linked in different sequences to encode a variety of actions. However, the systematic identification of these behavioral modules in mammals remains a significant challenge. Here we characterize how the three-dimensional (3D) poses exhibited by mice change over time during unrestrained behavior and observe that mouse pose dynamics are inherently divided into brief (circa. 400 millisecond) motifs of 3D motion. Computational modeling of these fast dynamics reveals that mouse behavior is composed from a set of reused modules, each a stereotyped

trajectory through pose space that is connected in time to other modules through predictable transitions. We observe that a striking behavioral response to a predator odor is implemented by simply changing transition probabilities between modules, while changing the physical shape of the experimental arena evokes new behavioral modules. The sub-second structure of behavior is also sensitive to changes in the genome and neural activity, enabling detection of subtle behavioral changes induced by heterozygous mutation of a gene or modest optogenetic stimulation. This work reveals that mouse body language _ like birdsong and natural language _ is built from identifiable components and is organized in a predictable fashion on sub-second timescales; deciphering this language establishes an objective framework for characterizing the influence of environmental cues, genes and neural activity on mouse behavior.

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Poster

090. Sensory and Motor Systems in Vertebrates

Location: Hall A

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Program#/Poster#: 90.18/AA26

Topic: F.04. Neuroethology

Support: VIEP-BUAP/Health 2015

CONACYT grants Nos. 243247 and 243333

Title: Dopamine agonist SKF 38393 increase syntactic grooming chains in high-yawning rats

Authors: *J. EGUIBAR[1], C. CORTES[2], A. TRUJILLO[3];
[2]Inst. of Physiol., [3]Biol. Sch., [1]Benemerita Univ. Autonoma de Puebla, Puebla, Pue., Mexico

Abstract: High-yawning (HY) subline of Sprague-Dawley rats yawned spontaneously more than the low-yawning (LY) under different environmental conditions. HY rats also had more grooming bouts and time spent in grooming behavior after when exposed to a novel environment or after wetting the fur. Grooming increased after systemic injection of D1 agonists such as SKF 38393. The aim of this study is to analyze the effects of SKF 38393 on syntactic grooming chains (SGC) in both sublines. All subjects were maintained under standard conditions with a light-dark cycle 12/12, free access to rodent pellets and water. At 100 days old, male subjects were injected s.c. with 16 mg/Kg of SKF 38393 and observed by 30 min between 11:00 to 13:00

h. The SGC were recorded using video camera and analyzed off-line by a blind observer using The Observer XT software. Statistical analysis did using ANOVA followed by Tukey´s test. The results showed that SKF 38393 increased the number and duration of SGC in both sublines ($P<0.05$). The D1 agonist produced a longer duration on SGC and hence the number of ellipses did around the vibrissae and ear areas in each SGC in both sublines being higher in HY ($P<0.05$). Finally SKF 38393 also increased the probability to complete SGC being greater in HY subjects ($P<0.05$). In conclusion, HY rats increased the number, mean duration of grooming bouts as well as the number of movements in the SGC after SKF 38393 with greater perseverance on the movements and higher velocity suggesting that HY subline is more anxious or of a perseverance of cleaning movements a characteristic of obsessive-compulsive disorder. Partly supported VIEP-BUAP/Health 2015 grants to JRE and CC and CONACYT grants Nos. 243247 and 243333 to JRE and MC, respectively. We deeply appreciate the support of Dr. Ygnacio Martínez, Vice-rector of Research

Disclosures: J. Eguibar: None. **C. Cortes:** None. **A. Trujillo:** None.

Poster

090. Sensory and Motor Systems in Vertebrates

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 90.19/AA27

Topic: F.04. Neuroethology

Support: NSF

AFOSR

ICB Army

Title: Speed vs accuracy: nervous systems tradeoffs using robust control

Authors: *Y. NAKAHIRA[1], J. C. DOYLE[2];
[2]Control and Dynamical Systems, [1]Caltech, Pasadena, CA

Abstract: Existing literature suggests neural networks evolved to optimize performance tradeoffs under various constraints, such as conduction velocity, energy consumption, signal to noise ratio, length of wiring, etc. We use robust control to develop a unified theory that connects channel capacity (information transfer between cells) and signaling delay (via conduction velocity) with computational performance in sensorimotor control tasks, and the resulting tradeoffs are consistent with the observed extreme heterogeneity in axon diameters. Delays,

feedback, noise, and dynamics are essential to the theory and are poorly treated in more familiar information theory and statistical physics. We model the nervous system as a feedback controller that attempts to maintain the body in a desired physical state despite disturbances, and neural fibers as communication channels between sensors of bodily and environmental states, controllers, and actuators (e.g. muscles). We first derive explicit formulas for robust performance of the feedback system given dynamic instability, signaling delay, actuator saturation, and the capacity of communication channels, where the performance is defined as the deviation from the desired state. Assuming that axonal fibers are contained in a region with fixed volume there is a tradeoff between signaling capacity (proportional to number of axons) and speed (proportional to radius for myelinated or sqrt(radius) for unmyelinated axon). These tradeoffs can be directly plugged into the robust performance formulas to find optimal capacities and delays as a function of the control objectives. The results show that, at one extreme, optimal robustness is achieved with minimum delay at the expense of low capacity when the environment is poorly known and fast reaction is required. In the other extreme, optimal robustness is achieved with large delay and signaling capacity when the environment is well known and slower action is acceptable. These results are consistent with but formalize for the first time the standard intuitions regarding the extreme heterogeneity in axon sizes needed to tradeoff speed with resolution in sensorimotor tasks.

Disclosures: **Y. Nakahira:** A. Employment/Salary (full or part-time):; California Institute of Technology. **J.C. Doyle:** None.

Poster

091. Biochemical Techniques

Location: Hall A

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Program#/Poster#: 91.01/AA28

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Melo Brain Research Project

Senacyt Grant

National Research System (SNI) of SENACYT

National Institute on Aging under Award Number R01AG039389

Title: Blood- and CSF-based biomarkers of Alzheimer’s disease, mild cognitive impairment and vascular dementia among Panamanians

Authors: *A. E. VILLARREAL[1], S. E. O'BRYANT[2], M. EDWARDS[3], S. GRAJALES[4], G. BRITTON[4];
[1]INDICASAT AIP, Panama, City of Panama, Panama; [2]Intrnl. Med., Inst. for Aging & Alzheimer's Dis. Research, Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; [3]Univ. of North Texas, North Texas, TX; [4]Ctr. de Neurociencias y Unidad de Investigación Clínica, Inst. de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), Panama, Panama

Abstract: The Latin American and Caribbean region is experiencing significant growth in the aging population. In Panama the geriatric population is gradually increasing as in the rest of the world. We are conducting a biomarker-based study to describe the demographic and biomarker profiles of Panamanians with Alzheimer's disease (AD), mild cognitive impairment (MCI) and vascular dementia (VaD) using a serum- and CSF-based biomarkers. Here we show the results of a cross-sectional analysis of aged individuals for blood-based (N=154) and CSF (N=51) biomarkers. The following measures were obtained: global cognition was measured with the Mini-Mental State Examination (MMSE), disease progression with the Global Deterioration Scale, and depression with the Geriatric Depression Scale 30-item version. Age, gender, education, ApoE ε4 allele frequency, diabetes diagnosis and obesity status were also analyzed. The blood-based biomarkers were measured using non-fasting serum samples and analyzed in duplicate via a multi-plex biomarker assay platform using ECL on the SECTOR Imager 2400A from Meso Scale Discovery. A biomarker profile was generated using random forest analyses. CSF biomarkers Aβ1-42, T-tau, and P-tau181 were analyzed using ELISA (enzyme-linked immunosorbent assay). For the blood-based biomarkers, preliminary results show that the profile for AD subjects was highly accurate, yielding an area under the receiver operating characteristic curve (AUC), sensitivity, and specificity of 0.94, 0.86 and 0.90, respectively. The diagnostic accuracy statistics did not change with the addition of age, gender, education and ApoE ε4 status. For the MCI and VaD groups, the accuracy was lower with AUC, sensitivity and specificity of 0.58, 0.30 and 0.71 (MCI) and 0.60, 0.80 and 0.25 (VaD), respectively. The expression of at least one copy of the APOEε4 allele differed among groups ($X2 = 17.7$, $p = 0.001$), with at least half of AD (57%) and MCI (50%) groups expressing one or two copies. For the CSF biomarkers, preliminary results show significant correlations between Aβ1-42, ($r = .323$, $p = 0.024$), T-tau ($r = .505$, $p = 0.000$), P-tau181 ($r = .409$ $p = 0.004$) and the MMSE. Other results show that levels of Aβ1-42 were significantly lower in AD patients vs. controls (mean [SD], 557 [246] pg/mL vs. 856 [305] pg/mL; $P = .001$). Levels of T-tau (mean [SD], 530 [253] pg/mL vs. 234 [95] pg/mL; $P<.001$) and P-tau181 were significantly higher in AD patients vs. controls (mean [SD], 88 [37] pg/mL vs. 44 [14] pg/mL; $P<.001$). This is the first study to assess both blood and CSF biomarkers for an elderly cohort of Panamanians and adds to a growing body of research on biomarkers of dementia among Hispanics.

Disclosures: A.E. Villarreal: None. **S.E. O'Bryant:** None. **M. Edwards:** None. **S. Grajales:** None. **G. Britton:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.02/AA29

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant NS 75013

NIH Grant NS 90455

Title: Development of boron-doped diamond microelectrodes for human use: pre-clinical animal trials

Authors: *E. N. NICOLAI[1], S. PAEK[1], P. H. MIN[1], J. R. TOMSHINE[2], M. P. MARSH[2], M. L. SETTELL[1], S.-Y. CHANG[1], K. E. BENNET[2], D. JANG[3], F. S. MANCIU[4], K. H. LEE[1]; [1]Dept. of Neurologic Surgery, [2]Div. of Engin., Mayo Clin., Rochester, MN; [3]Dept. of Biomed. Engin., Hanyang Univ., Seoul, Korea, Republic of; [4]Dept. of Physics, Univ. of Texas, El Paso, TX

Abstract: Introduction: Fast scan cyclic voltammetry (FSCV) is an electrochemical technique used to measure electroactive molecules. This technique has proven powerful in providing an understanding of the real-time action of neurotransmitters such as dopamine and adenosine *in vivo*. The ability to measure neurotransmitters in humans would provide valuable biomarkers for neurosurgical techniques such as deep brain stimulation (DBS). Carbon fiber microelectrodes (CFM) are the current standard for *in vivo* FSCV recordings; however, this technology is not suitable for human use, as CFMs are fragile and degrade over time. Here we describe the pre-clinical testing of a boron-doped diamond (BDD) microelectrode in animal models of DBS for the ultimate goal of use in human patients. Method: BDD microelectrodes were designed and produced in-house. The Mayo-developed WINCS Harmoni was used to produce an FSCV waveform scanned from -0.4 to 1.45 V and back at 400 V/s every 100 ms for all experiments. Sensitivity to dopamine was assessed in multiple rodents via medial forebrain bundle stimulation and FSCV recording in the caudate putamen. Stimulation-evoked signals were obtained before and after Nomifensine, a dopamine reuptake inhibitor, injection in order to verify recording of dopamine. Sensitivity to adenosine was assessed in multiple rodents, multiple swine, and a single non-human primate via mechanical stimulation of the caudate putamen and thalamus while recording FSCV in the same location. Signals obtained were assessed by the presence of oxidation current at the known oxidation potentials for adenosine, 1.4 and 1.0 V. Longevity *in vivo* of BDD microelectrodes was compared to that of CFMs by using the same electrode of each

type multiple times in multiple animal experiments. Results: The BDD microelectrodes demonstrated the ability to measure dopamine in rodents and adenosine-like signals in rodents, swine, and a non-human primate. Compared to CFMs used in the same series of rodent experiments, BDD microelectrodes appeared to have degraded at a slower rate and maintained clearer, more robust signal detection. Finally, multiple CFM tips broke during experiments, while all of the BDD microelectrodes remained intact. Conclusion: The ability of BDD microelectrodes to measure electrically stimulated dopamine release and mechanically stimulated adenosine-like release *in vivo* supports their efficacy for use in detecting biomarkers related to DBS. Importantly, these results support the sensitivity, longevity, and safety of BDD microelectrodes for use in future human studies.

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Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.03/AA30

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: AB oligomers and AD

Authors: *J. KALININA[1], M. S. MICHENER[1], B. E. SMITH[1], E. PARKER[1], J. A. STONE[1], E. VAN MAANEN[2], G. WILCOCK[3], D. SMITH[3], D. WARDEN[3], C. L. MASTERS[4], Q.-X. LI[4], C. J. FOWLER[4,2], C. J. WINROW[1], M. J. SAVAGE[1];
[1]Late Discovery and AD, Merck & Co., West Point, PA; [2]LAP&P consultants, Leiden, Netherlands; [3]Oxford Univ., Oxford, United Kingdom; [4]Melborne Univ., Melborne, Australia

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative dementia. Merck's β-secretase therapy against AD is currently undergoing Phase 3 clinical trials and shows dramatic reductions in Aβ monomer levels. With the recent emergence of Aβ oligomers as the most toxic Aβ species in AD, our work has focused on deciphering the different roles of Aβ oligomers in AD: (1) by establishing a sensitive, robust and automated assay for their scarce (sub-pg/mL) detection in human CSF, and (2) demonstrating their use as a diagnostic, and a marker in evaluating drug/oligomer pharmacodynamics in preclinical/clinical models of APP processing. To achieve the former, we used Singulex' Erenna technology to develop Aβ oligomer-specific CSF assay that can reliably discriminate AD vs. controls (*J.Neurosci*. **2014**,

34(8):2884-97). In our recent work, we are developing a prototype oligomer assay using Quanterix Simoa technology, which will is hoped to improve throughput, fully automate the assay, and reduce sample volumes. Using the Singulex assay and working collaboratively with several academic institutions (University of Melbourne, Oxford University), we have also explored the correlation of CSF oligomers with cognitive decline in cross-sectional/longitudinal AD and Control cohorts (AIBL, OPTIMA). Finally, in our effort to identify Aβ oligomers as a pharmacodynamic biomarker in response to secretase inhibition, we used cisterna magna ported-rhesus preclinical model in a four-way crossover design. Treatment with secretase inhibitors (BACE, GSi or vehicle) resulted in a significant (5-fold) reduction in oligomers in rhesus CSF in a time-/dose-dependent fashion, enabling a revised model of rhesus CNS APP processing that includes a role for oligomers. Together, these findings highlight potential utility of Aβ oligomers as diagnostic and prognostic tools for human AD; and, importantly, as a novel marker of pharmacodynamic response to secretase inhibitor therapy. Future work is planned to measure oligomer levels in clinical trials' CSF samples of BACEi.

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Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.04/AA31

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Evaluating transcytosis of bivalent and monovalent transferrin receptor antibodies *in vitro*

Authors: *F. LIU, S.-H. TSCHANG, J. PEREIRA, J. CROY, J. LU, R. DEMATTOS, M. RACKE;
Eli Lilly & Co., Indianapolis, IN

Abstract: The blood brain barrier (BBB) restricts entry of antibodies to the CNS to a mere 0.1 - 0.2%. Transferrin receptor (TfR) antibodies have been explored as carriers to deliver therapeutic antibodies across the BBB via receptor mediated transcytosis (RMT). Previous studies demonstrated that binding mode influences RMT and therefore brain exposure and drug potency. In order to better understand the impact of antibody valence on the efficiency of RMT, we examined several TfR antibodies as either monovalent or bivalent constructs in *in vitro* BBB

models. We characterized both human hCMEC/D3 and murine bEND.3 brain endothelial cells (EC) grown in transwells to assure appropriate conditions to test RMT. Then we used a pulse/chase method to allow uptake of antibodies into the monolayer and analyzed their internalization and subsequent trafficking throughout the system. We evaluated anti-human TfR antibodies mAB128.1 and MEM189 in both monovalent and bivalent forms in the hCMEC/D3 cells. The monovalent versions of antibody were internalized by the cells at half the level of their bivalent counterparts. For both monovalent and bivalent mAB128.1, the percentage of the antibody in the basolateral chamber compared to what was taken up by cells, was similar. However, for the monovalent MEM189 this percentage was significantly higher than the bivalent form. This suggests more robust transport of monovalent MEM189 out of the EC once internalized. In contrast to the human antibodies, monovalent and bivalent constructs of anti-mouse TfR antibody Mab8D3 entered murine bEND.3 cells to similar levels. Like the monovalent MEM189, monovalent Mab8D3 shows a higher percentage of externalization than the bivalent antibody. Our data demonstrates the utility of two brain endothelial cell lines in testing the design and activity of TfR antibodies to enhance BBB penetration of large molecule therapeutics.

Disclosures: **F. Liu:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **S. Tschang:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **J. Pereira:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **J. Croy:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **J. Lu:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **R. Demattos:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **M. Racke:** A. Employment/Salary (full or part-time):; Eli Lilly & Co..

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.05/AA32

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIDA DA027969

NSF CHE1151264

Title: Cocaine induced glucose and dopamine fluctuations simultaneously recorded at single striatal locations using fast-scan cyclic voltammetry

Authors: *S. SMITH[1,2], W. GARRISON[1], C. LEE[1], A. KOMSA[1], L. SOMBERS[1];
[2]Chem., [1]North Carolina State Univ., Raleigh, NC

Abstract: Cocaine, a drug that is commonly abused, elicits a well-characterized and robust dopamine release in the striatum. Even cues that predict cocaine availability elicit striatal dopamine release, and it is this chemical signaling that is thought to underlie cocaine's reinforcing effects. However, the metabolic effects of cocaine administration are unclear, as direct attempts to investigate this question have produced controversial results. In fact, it is unclear how glucose, a key metabolic marker, fluctuates on a second-by-second basis in normal brain function, despite the fact that it serves as the principal energy source of the brain. This work aims to simultaneously quantify dynamic fluctuations of glucose and dopamine at single recording sites in the rat striatum using glucose oxidase-modified carbon-fiber microelectrodes coupled with background-subtracted fast-scan cyclic voltammetry, under basal conditions and in response to cocaine administration. This approach provides chemical selectivity, revealing sub-second dynamics of both chemicals at a single recording site to directly demonstrate how robust striatal dopamine release mediates local metabolic processes. An understanding of this interaction promises to inform therapeutic strategies for treating drug addiction and related disorders.

Disclosures: S. Smith: None. **W. Garrison:** None. **C. Lee:** None. **A. Komsa:** None. **L. Sombers:** None.

Poster

091. Biochemical Techniques

Location: Hall A

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Program#/Poster#: 91.06/AA33

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Label-free method for characterization of diprenorphine binding to active and inactive state u-opioid receptors using LC-MS/MS detection

Authors: *T. EESSALU[1], M. JOHNSON[2], L. MARTIN[2], K. BURRIS[3], V. BARTH[1], T. WIERNICKI[3];
[1]Tailored Therapeutics- Neurosci., [2]Neurosci. Disc. Res., [3]DCRT, Eli Lilly & Co, Indianapolis, IN

Abstract: We report a simple highly sensitive quantitative label-free method for detecting direct receptor-ligand binding interactions using LC-MS/MS detection. This label-free method was

used to determine biophysical properties (koff, Kd, Bmax) of the non-selective opioid receptor binding ligand diprenorphine to cell membranes overexpressing human μ-opioid receptors. The equilibrium binding constant (Kd), number of binding sites (Bmax) and ligand dissociation rate constant (Koff) were determined under buffer conditions that promote both G-protein receptor active stat (coupled) and inactive state (uncoupled). Using the label-free direct binding method we determined that the results agree to published data using the indirect radiolabel tracer method. In the presence of GDP and sodium chloride the μ-opioid receptors were uncoupled from G-proteins and we saw the expected shift of the receptor equilibrium to the inactive state by a 20 fold decrease in equilibrium binding affinity (Kd) while the total number of available binding sites (Bmax) remained unchanged. We also determined that when the dissociation binding profile for diprenorphine μ-opioid receptors was performed under uncoupled buffer conditions a biphasic curve was seen with both fast and slow components. However, when dissociation experiments were performed under coupled buffer conditions, a single slow dissociation rate was seen. This LC-MS direct binding assay greatly enhances the speed at which receptor ligand candidates can be biophysically characterized, when compared to radiolabeling prospective candidate ligand molecules. The direct LC-MS binding detection technique now makes it possible to assess receptor binding interactions for agonists (full, partial, inverse), antagonists (competitive or non-competitive), or allosteric (positive or negative) modulators.

Disclosures: **T. Eessalu:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **M. Johnson:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **L. Martin:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **K. Burris:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **V. Barth:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **T. Wiernicki:** A. Employment/Salary (full or part-time):; Eli Lilly & Co.

Poster

091. Biochemical Techniques

Location: Hall A

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Program#/Poster#: 91.07/AA34

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R21AG045637

NUCATS Grant FY2104-2015

Title: An ultrasensitive immunoassay detects soluble Aβ oligomers in Tg mice prior to memory loss

Authors: E. N. CLINE[1], K. L. VIOLA[1], M. G. ROLLINS[1], S. N. MOHAMMAD[1], L. R. ZIESKE[2], *W. L. KLEIN[1];
[1]Neurobio., Northwestern Univ., Evanston, IL; [2]Singulex, Inc., Alameda, CA

Abstract: Background: The recent success of a Phase 1b clinical trial (antibody aducanumab; Biogen Idec) substantiates the hypothesis that aggregated forms of Aβ have a key role in Alzheimer's disease (AD) pathogenesis. Because amyloid beta oligomers (AβOs) are widely regarded as the aggregated form of Aβ responsible for AD onset, the therapeutic efficacy of aducanumab may come from targeting AβOs rather than amyloid plaques. AβOs accumulate early in AD and experimentally cause memory dysfunction and the major cellular pathologies associated with AD (*e.g.*, tau abnormalities, synapse loss, oxidative damage, *etc.*). An important resource currently unavailable to clinicians and researchers is an ultrasensitive assay for detection of AβO species at AD-relevant concentrations. Such an assay would have potential application to early diagnosis and therapeutics. **Methods:** We have developed an *ultrasensitive AβO immunoassay* (lower limit of quantification: 0.10 pg/mL = 20 fM) utilizing the research use only Erenna® Immunoassay Platform (Singulex) and a *highly AβO-specific human monoclonal antibody* (ACU-193; Acumen). This antibody is unique compared to Aβ-targeting antibodies that have gone to clinical trials in that it has little to no affinity for monomers or fibrils.
Results: With this assay, we have detected 3-10 fmol AβOs per mg total protein in brain extracts of 5xFAD Tg mice at 3.5 months, *prior to the onset of memory loss*. This preliminary data was obtained from 6 Tg mice and 3 wt littermates. At 24 months of age, AβO levels in Tg mice are increased another 10-fold. **Conclusions:** This preliminary data provides further evidence for the early accumulation of AβOs in AD. Future applications of this immunoassay include: (1) AβO detection in human brain extracts and, potentially, concentrated CSF; and (2) when combined with HPLC-SEC, characterization of the size distribution of AβOs throughout AD progression. Detection of specific populations of AβOs will fill a gap in our knowledge of the native structure of species prevalent over the course of AD and may show greater diagnostic potential than detection of whole AβO populations.

Disclosures: E.N. Cline: None. **K.L. Viola:** None. **M.G. Rollins:** None. **S.N. Mohammad:** None. **L.R. Zieske:** None. **W.L. Klein:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acumen Pharmaceuticals, Inc..

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.08/AA35

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Anticonvulsant effects of non-selective nonsteroidal anti-inflammatory drug and cyclooxygenase-2 selective inhibitor in the zebrafish seizure model

Authors: *P. G. BARBALHO;
Univ. of Campinas, Campinas, Brazil

Abstract: Introduction: Epilepsy is a common neurological disorder characterized by recurrent spontaneous seizures. Cyclooxygenase-2 (COX-2) is an enzyme that converts arachidonic acid into prostaglandins and it has been suggested that this enzyme can play a role in epilepsy. The present study was carried out to investigate the effect of the non-selective NSAID and COX-2 selective inhibitor (indomethacin and SC-236, respectively) on seizure onset latency and number of seizure behavior in the zebrafish seizure model. **Methods:** zebrafish were maintained according to standard procedures. All experiments procedures were approved by the Ethic Animal Committee. Seven days post-fertilization (dpf) larvae were placed individually in a 24 well-plate containing 15mM PTZ for 20 minutes. During PTZ exposure, the latency of seizure onset (n=10) and number of seizures (n=5) were analyzed. Latency was considered when the animal was exposed to PTZ until it achieves stage 3 (loss of posture). Six dpf zebrafish larvae were incubated in 307μM indomethacin solution or 1μM SC-236 solution (0.1% DMSO) in petri dishes for 24 hours and after that, at 7dpf, they were exposed to 15mM PTZ as described above. Data are presented as mean values ± standard error of mean (SEM). Statistical analyses were performed by Mann-Whitney test with $p \leq 0.05$ for a significant difference between groups, using the GraphPad Prism. **Results:** Indomethacin and SC-236 increased latency to seizure onset (n=10; $p<0.001$ and $p=0.001$, respectively) and reduced the number of seizure behavior when compared to animal from SG (n=10; $p=0.004$ and $p=0.007$, respectively). The mean±SEM for the latency to seizure onset (give in minutes) was: SG 2.2±0.25; 307μM indomethacin+SG 5.2±0.27; 1μM SC-236 3.9±0.37. The mean±SEM of number of seizure obtained for each group was: SG 34.4±6.2; 307μM indomethacin+SG 9.4±1.5; 1μM SC-236 3.0±0.7. **Conclusion:** Indomethacin and SC-236 treatment prior to PTZ-induced seizure increased the latency to seizure onset and significantly decreased the number of seizures during PTZ exposure, showing that both treatments presented an anticonvulsant effect in this model. Our findings support evidence that zebrafish is a valuable model for further investigations of the main role of inflammation in seizure as well as it is a valuable model for anti-inflammatory screening of compounds that are potentially therapeutic for seizures suppression. Support: FAPESP

Disclosures: P.G. Barbalho: None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.09/AA36

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Multiplex immunoassay detection of neurodegenerative and cytokine biomarkers in cerebrospinal fluid

Authors: *A. J. SAPORITA, J. MISTRY, J. HWANG;
EMD Millipore, Saint Charles, MO

Abstract: Progressive neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's Disease (AD) affect millions worldwide and are becoming more prevalent as our population ages. Monitoring protein biomarkers in cerebrospinal fluid (CSF) of patients with these neurological disorders may be highly beneficial to understanding disease progression. Whereas biomarkers of AD have been established, the identification of reliable protein biomarkers to aid in the diagnosis of PD and the characterization of PD progression has been elusive. To add further complexity, some biomarkers are general indicators of neurodegeneration but cannot be used to distinguish AD and PD. For example, many cytokines are general biomarkers of neuroinflammation associated with these neurological diseases. Simultaneous monitoring of several protein biomarkers, using a multiplexed panel, may further our understanding of disease progression. We have developed a multiplex immunoassay to eight protein biomarkers of neurological disease: alpha-synuclein, glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), angiogenin, transglutaminase 2 (TGM2), prion protein, UCHL1, and DJ-1. Notably, several of these proteins have been linked to PD. This assay is compatible with analytes from the MILLIPLEX®MAP Human Cytokine Panel I, allowing for rapid customization to enable simultaneous detection of both the cytokines and the other neurological disease biomarkers. Using the customized 20-plex immunoassay, we measured the expression of these 8 neurological disease biomarkers and 12 cytokines in CSF from PD patients, AD patients, and healthy controls. Angiogenin and NSE showed increased expression in PD samples relative to controls, while GFAP, NSE, UCHL1 and prion protein were elevated in AD samples. Several cytokines were detectable in CSF, including IL-4, IL-5, and VEGF, which showed increases in both PD and AD samples compared to controls. Increases in IFN-gamma and TNF-alpha also reached statistical significance in the AD group compared to controls. Angiogenin was the only analyte in our assay to exhibit selective elevation in PD CSF but not AD CSF. This study demonstrates the value of using customizable multiplex technology to evaluate multiple biomarkers of neuroinflammation and neurodegeneration.

Disclosures: **A.J. Saporita:** A. Employment/Salary (full or part-time):; EMD Millipore. **J. Mistry:** A. Employment/Salary (full or part-time):; EMD Millipore. **J. Hwang:** A. Employment/Salary (full or part-time):; EMD Millipore.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.10/AA37

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Crossing the blood-cerebrospinal fluid barrier in the mouse choroid plexus with an engineered receptor/ligand system

Authors: *H. R. MENDEZ-GOMEZ[1,2], A. GALERA-PRAT[3,4], C. MEYERS[1,2], J. SINGH[1,2], W. CHEN[1,2], M. CARRION[3,4], N. MUZYCZKA[1,2];
[1]Mol. Genet. and Microbiology, Univ. of Florida, Gainesville, FL; [2]Powell Gene Therapy Ctr. and UF Genet. Inst., Gainesville, FL; [3]Inst. Cajal, Madrid, Spain; [4]Inst. Madrileño de Estudios Avanzados en Nanociencia, Madrid, Spain

Abstract: Crossing the blood-brain and the blood-cerebrospinal fluid barriers are one of the fundamental challenges in the development of new therapeutic molecules for brain disorders because they prevent entry of most drugs from the blood into the brain. However, some large molecules, like the protein transferrin, cross these barriers using a specific receptor that transports them into the brain. Mimicking nature, we engineered a receptor/ligand system to cross the brain barrier by combining the human transferrin receptor with the cohesin domain from Clostridium thermocellum, and we tested it in the choroid plexus of the mouse brain with a dockerin ligand. By expressing our receptor in choroidal ependymocytes, which are part of the blood-cerebrospinal fluid barrier, we found that our systemically administrated ligand was able to bind to the receptor and accumulate in ependymocytes, where some of the ligand was transported from the blood side to the brain side.

Disclosures: **H.R. Mendez-Gomez:** None. **A. Galera-Prat:** None. **C. Meyers:** None. **J. Singh:** None. **W. Chen:** None. **M. Carrion:** None. **N. Muzyczka:** None.

Poster

091. Biochemical Techniques

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Program#/Poster#: 91.11/AA38

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: 1U01NS090455-01

Title: Differentiation of analytes using multiple fast-scan cyclic voltammetry

Authors: *D. KIM[1], Y. OH[1], H. SHIN[1], C. PARK[1], K. E. BENNET[2], I. KIM[1], D. JANG[1], K. H. LEE[2];
[1]Hanyang Univ., Seoul, Korea, Republic of; [2]Mayo Clin., Rochester, MN

Abstract: In this study, we present a new method that is similar to paired-pulse voltammetry (PPV) but uses multiple pulses to calculate the kinetic properties of neurochemicals. We call this technique multiple fast-scan cyclic voltammetry (MFSCV), in which multipulse (10 pulses per scan) cyclic voltammetry is done at various voltages. We used the conventional triangle waveform (from -0.4 volt to +1.0 volt and back down to -0.4 volt, at a scan rate of 1000 V/s) and a 10Hz repetition time for our experiments. For practical applications of MFSCV, it can be possible to change the scan waveform, scan rate, scan repetition time, and each scan interval. These successive cyclic voltammograms have a rapidly decreasing patternbecause the adsorption time is decreasing rapidly. This means that the amount of adsorbed chemicals has a lower saturation with higher scan repetition rates at the same concentration of the neurotransmitter. As a result, in this study we have derived a theoretically decreasing pattern of dopamine (DA) using MFSCV and produced background- subtracted voltammograms, in which their differences can be fitted in the exponential equation that has been expanded to all voltages for making a new type of pseudo color maps. These maps are composed of two features: the kinetic maps (K-maps) that show the specific kinetic characteristics of a particular analyte and the concentration maps (A-maps) for said analyte. Using this method, we can get the conventional information and the desorption-reduction related constant maps at the same time. Lastly, we applied the MFSCV method to the three other electroactive species groups: catecholamines, indolamines and ascorbic acid (AA). These three groups each have different the desorption-reduction related constant characteristic. This flow cell experiment showed the coincidence with our derived theoretical values, since MFSCV values are affected by the previous iteration of the redox amount and ratio at the carbon fiber microelectrode surface.

Disclosures: **D. Kim:** None. **Y. Oh:** None. **H. Shin:** None. **C. Park:** None. **K.E. Bennet:** None. **I. Kim:** None. **D. Jang:** None. **K.H. Lee:** None.

Poster

091. Biochemical Techniques

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Program#/Poster#: 91.12/AA39

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: 1U01NS090455-01

Title: Measurement of dopamine absolute concentration using multiple fast-scan cyclic voltammetry

Authors: *Y. OH[1], D. KIM[1], H. SHIN[1], C. PARK[1], K. E. BENNET[2], I. KIM[1], D. JANG[1], K. H. LEE[2];
[1]Hanyang Univ., Seoul, Korea, Republic of; [2]Mayo Clin., Rochester, MN

Abstract: Neurotransmitters are endogenous chemicals that transmit signals between neurons across a synapse. This neurochemical transmission is a significant biomarker for understanding neuro-functional mechanism in the brain along with electrophysiological signals. Many techniques have been proposed to measure neurotransmitter concentration in the brain. Fast-Scan Cyclic Voltammetry (FSCV) is one such method thatmeasures neurotransmitters by detecting fine current occurring in the brain by applying voltage through a carbon fiber microelectrode (CFM). This technique has been used experimentally to measure chemical fluctuation within milliseconds in mammals. Dopamine (DA) is one metabolite measureable using FSCV. Nonetheless, because the background signal in FSCV is relatively large and unstable, tonic concentration of DA cannot be separated from the background signal. However, Heine, et. al., recently suggested a fast-scan controlled-adsorption voltammetry (FSCAV) technique for quantifying tonic levels of dopamine in the brain. In this research, we propose an optimized FSCV technique based on adsorption, desorption reaction within minimized CFM double layer to extract the absolute value of DA. By minimizing background signal and maximizing DA response in FSCV, we could detect absolute DA signal without utilizing the background subtraction method. Then, we could estimate specific concentration of DA using the extracted characteristic value from basic buffer. Unlike FSCAV, we could measure the absolute value of DA with clear features of oxidation and reduction of DA in striatum of a rat brain with minimized background effect.

Disclosures: Y. Oh: None. **D. Kim:** None. **H. Shin:** None. **C. Park:** None. **K.E. Bennet:** None. **I. Kim:** None. **D. Jang:** None. **K.H. Lee:** None.

Poster

091. Biochemical Techniques

Location: Hall A

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Program#/Poster#: 91.13/AA40

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Engineering an "armor-plated" horseradish peroxidase (HRP) for connectomic studies

Authors: *M. G. PAEZ SEGALA[1], R. FETTER[2], J. SIMPSON[3], L. LOOGER[4];
[1]Looger Lab., Janelia Farm, HHMI, Ashburn, VA; [2]Dr Richard Fetter, [3]Dr. Julie Simpson, [4]Dr. Loren Looger, Janelia Res. Campus, HHMI, Ashburn, VA

Abstract: In 1810, Planche 1reported that a tincture of guaiacum developed a stronger color when a piece of fresh horseradish root was soaked in it. During the next two centuries, horseradish peroxidase (HRP), responsible for the color change in Planche's experiment, was discovered, characterized and subsequently used extensively as a staining agent in diverse applications. In recent years the labeling of neurons and subcellular structures with subsequent visualization at nanometer resolution using different electron microscopic imaging strategies, such as focused ion beam milling block-face scanning electron microscopy (FIB-SEM) and transmission EM (TEM) (c.f. 2,3)_ENREF_1 has become a major workhorse in anatomy. Sample preparation for these applications usually incorporates HRP-generated H2O2-catalyzed polymerization of diaminobenzidine (DAB), which is then made electron-dense by subsequent reaction with osmium tetroxide (OsO4). These DAB/osmium deposits are easily visualized by electron microscopy or X-ray tomography. When using large-volume samples a compromise must be found between sample preparation for excellent ultrastructural preservation and the ability of HRP to catalytically produce a robust DAB product. Generally with higher concentration and duration of glutaraldehyde fixation, HRP's ability to catalyze DAB polymerization may be weakened or eliminated due to inactivation of the enzyme. In this work we report the rational design and molecular engineering of HRP's surface-reactive amino acids to decrease the sensitivity to aldehyde fixation, with the goal to permit robust enzymatic activity and ultrastructural preservation for electron microscopy. "Killer applications" include targeted staining of genetically-specified neurons, glia, or organelles in brains prepared for ultra-high-quality EM reconstruction for connectomics. References: 1Planche. Note sur la sophistication de la résine de jalap et sur les moyens de la reconnaître. Bull Pharmacie 2, 578-580 (1810). 2Sonomura, T. et al. Correlative analysis of immunoreactivity in confocal laser-scanning microscopy and scanning electron microscopy with focused ion beam milling. Frontiers in neural circuits 7, 26, doi:10.3389/fncir.2013.00026 (2013). 3Atasoy, D. et al. A genetically specified connectomics approach applied to long-range feeding regulatory circuits. Nature neuroscience 17, 1830-1839, doi:10.1038/nn.3854 (2014).

Disclosures: **M.G. Paez Segala:** None. **R. Fetter:** None. **J. Simpson:** None. **L. Looger:** None.

Poster

091. Biochemical Techniques

Location: Hall A

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Program#/Poster#: 91.14/AA41

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Development of a surface plasmon resonance assay for characterization of small molecule binding kinetics and mechanism of binding to kynurenine 3-monooxygenase

Authors: *S. PODA[1,2], M. KOBAYASHI[2], R. NACHANE[2], V. MENON[1], A. S. GANDHI[1], D. P. BUDAC[1], G. LI[1], B. M. CAMPBELL[1], L. TAGMOSE[1];
[1]Neuroinflam. biology, Lundbeck USA, Paramus, NJ; [2]Structural Biol., ZoBio. BV, Leiden, Netherlands

Abstract: When developing lead compounds against a validated drug target, the traditional drug discovery paradigm has focused primarily on optimizing drug-target interactions via the measurement of IC_{50} or K_i. However, it is recognized that efficacy and selectivity may also be driven by drug-target kinetics and the mechanism of binding an overlooked dimension in the conventional structure-activity relationships. In particular, drug-target residence time, which is defined as the reciprocal of the dissociation rate constant, has been shown to be a promising early stage indicator of *in vivo* drug activity. Kynurenine 3-monooxygenase (KMO), a pivotal enzyme in the kynurenine pathway was implicated as a potential therapeutic target for treating neurodegenerative and psychiatry disorders. Recently, RapidFire LC/MS/MS was reported as a sensitive, label-free, and direct method for monitoring the conversion of kynurenine to 3-hydroxykynurenine catalyzed by KMO. Previously, KMO assays based on absorbance of NADPH or release of tritiated water were described. However, none of these methods are suitable for the determination of the binding kinetics of compounds to KMO. We describe here a surface plasmon resonance assay which delivers both kinetic and the mechanism of binding data, enabling a detailed characterization of compound interactions with the KMO enzyme in real time.

Disclosures: **S. Poda:** None. **M. Kobayashi:** None. **R. Nachane:** None. **V. Menon:** None. **A.S. Gandhi:** None. **D.P. Budac:** None. **G. Li:** None. **B.M. Campbell:** None. **L. Tagmose:** None.

Poster

091. Biochemical Techniques

Location: Hall A

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Program#/Poster#: 91.15/AA42

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Gamma amino-butyric-acid measurement by an electrochemiluminescence method in small samples with high sensitivity

Authors: *J. C. SALAZAR SANCHEZ, A. MORALES-VILLAGRAN;
Dept. de Biología Celular y Mol., Univ. De Guadalajara, Zapopan, Mexico

Abstract: Gamma amino-butyric-acid measurement by an electrochemiluminescence method in small samples with high sensitivity. J. C. Salazar Sánchez and A. Morales Villagrán Gamma amino-butyric-acid (GABA) is the main inhibitory neurotransmitter in the central nervous systems. Alteration in its concentration is associated with several neurological disorders like Epilepsy, Parkinson, Alzheimer and Huntington disease. It has an important role in intercellular communication both in central nervous system as well as in other tissues. A complete characterization of GABA in several physiological and pathological processes requires a reliable quantification of this compound. Several methods have been developed with the aim to quantify GABA based on the instrumentation available, sensitive required, and organ/tissue analyzed. Most of these methods mainly use HPLC coupled to fluorescence and electrochemical detection. These methods requires a minimal volume for each sample and the sensitivity range is in micromolar concentration, which in some cases they do not reach the desired sensitivity for specific samples. The alternative suggested here is based on the use of enzymatic reactors that produce electrochemiluminescence signal. This is obtained using the well know GABAse complex that produces Nicotinamide adenine dinucleotide reduced form (NADH) as well as glutamate (Glu) among others products. Additional enzymatic reactors to determine the NADH and Glu are coupled. These are NADH oxidase and Glutamate oxidase that generate hydrogen peroxide, which is quantified in an electrochemiluminscence way in the presence of luminol. The luminescence obtained was proportional to the GABA concentration, for NADH, the calibration curves had a polynomic behavior with a R^2 value ≥ 0.97 in a nanomolar range (3-1000). Using Glu measurement derived from GABA, the sensitivity obtained was in the nanomolar range (50-1000) with a linear R^2 value ≥ 0.95. These alternatives could be used to determine this neurotransmitter in small volume samples and the main advantage is that a big number of samples can be analyzed in a short time.

Disclosures: J.C. Salazar Sanchez: None. **A. morales-villagran:** None.

Poster

091. Biochemical Techniques

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: DA033533

DA037294

AA023183

AA022082

DA036241

AA007456

DA032898

Title: Afferent-specific neurochemical profiling of multiple glutamatergic inputs to the nucleus accumbens by combined optogenetics and microdialysis application

Authors: *N. SUTO[1], A. MATZEU[1], A. LAQUE[1], M. W. BUCZYNSKI[1], S. AZUMA[2], T. KERR[1], D. WATRY[1], R. MARTIN-FARDON[1], L. H. PARSONS[1], F. WEISS[1], P. P. SANNA[1], T. C. JHOU[3];
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Abstract: Optogenetics is widely adopted in many branches of neuroscience. This rapidly evolving technology takes the advantage of light-gated receptors to manipulate genetically targeted biological systems with progressively increasing degree of cell-type and circuit level specificity. However, the neurochemical impacts of optogenetic manipulation largely remain uncharacterized and are only indirectly inferred by the effects of secondary pharmacological manipulation (antagonists) on electrophysiological and/or behavioral responses. In the present study, the neurochemical impacts of optogenetic stimulation of glutamatergic inputs to the nucleus accumbens (NAc) - neurochemical signal thought to encode reward-associated cues, and thus mediate reward-seeking (motivated) behaviors - were directly monitored by brain microdialysis in anesthetized rats. In NAc, the prototypical excitatory transmitter glutamate is known to trigger post-synaptic action potential in intrinsic neural population as well as pre-

synaptic release of diverse neurochemicals. Based on a previous report (Britt et al., 2012, Neuron, 76, 790-803), distinct glutamatergic afferents originating in the medial prefrontal cortex (mPFC), basolateral amygdala (BLA) or ventral hippocampus (vHipp) were examined in separate groups of rats. In all cases, afferent-specific optogenetic stimulation of distinct glutamatergic inputs and microdialysis sampling of extracellular neurochemicals were both conducted in the same brain region - either core or shell of NAc. Neurochemical contents of the dialysate samples were resolved by high performance liquid chromatography, and detected by a conventional electrochemical method (Eicom) as well as by a high-throughput mass-spectrometry method (Song et al., 2012, Anal. Chem., 84, 412-9). Analytes of interest included glutamate, GABA, dopamine, norepinephrine, serotonin, acetylcholine, histamine, adenosine, serine, glycine, and taurine. Support: DA033533 (N.S), DA037294 (N.S.), AA023183 (N.S.), DA035865 (M.W.B.), DA033344 (R.M.F.), AA007456 (L.H.P.), AA022082 (F.W.), DA036241 (P.P.S.), DA032898 (T.C.J.), DA037327 (T.C.J.).

Disclosures: **N. Suto:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Eicom. **A. Matzeu:** None. **A. Laque:** None. **M.W. Buczynski:** None. **S. Azuma:** A. Employment/Salary (full or part-time):; Eicom. **T. Kerr:** None. **D. Watry:** None. **R. Martin-Fardon:** None. **L.H. Parsons:** None. **F. Weiss:** None. **P.P. Sanna:** None. **T.C. Jhou:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Eicom.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.17/AA44

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NSF grant EPS-1003907

NASA WVSGC Undergraduate Fellowship (L.E.H.)

Title: Fibrin-based microenvironments for *in vitro* studies of adult neurogenesis

Authors: *L. HAGER[1], A. CLARK[2], E. PRICE[2];
[2]Biol., [1]Marshall Univ., Huntington, WV

Abstract: Adult neurogenesis is an on-going process in the mammalian brain and our laboratory is interested in harnessing adult neural stem cells for therapeutic applications. The subventricular

zone (SVZ) is a niche of neural progenitors whose normal function is to repopulate olfactory bulb neurons. These stem cells migrate from the SVZ to their destination via the rostral migratory stream (RMS). The long term goal of this research is to develop implantable fibrin-based hydrogels which are capable of forming a de novo migratory stream that steers migrating neural cells from their usual path into a new non-neurogenic region. For this approach to be successful, the matrix must provide the appropriate cues that signal migration, axonogenesis and dendritogenesis, integration and function. In the present work, we prepared small fibrin cylinders containing covalently crosslinked neurotrophins selected based on their predicted functional impact on adult neural progenitor cell maturation. We have incorporated VEGF and GDNF, alone, in combination, or sequentially, into these cylinders which were placed into tissue culture wells seeded with cultured neural progenitor cells. When VEGF and GDNF were both present, mature NF160+ neurons with complex neurites were seen growing along the matrix. Interestingly, when only VEGF or GDNF was present, cells were also observed migrating along the cylinder but lacked complex neurites. When VEGF was crosslinked into the cylinders and GDNF was added to the culture on Day 3, complex NF160+ neurites were again observed, suggesting that VEGF was required to render the neural progenitors responsive to GDNF. These findings lead to a model of neurogenesis where there is a temporal requirement for factors where specific neurotrophins are required to induce cellular responsiveness to subsequent factors. To provide additional mechanistic information that will lead to an engineered implant that can recapitulate the RMS, we generated a cylindrical matrix consisting of two smaller cylinders, one inside of the other. The inner cylinder was cast containing neural progenitor cells and the outer cylinder contained vascular endothelial cells. After several days in culture, the inner fibrin cylinder was degraded by the neural cells, which themselves migrated along the newly formed lumen of the outer cylinder, exhibited complex neurites and formed a three dimensional architecture with the endothelial cells. The findings from these *in vitro* experiments will provide guidance in the development of cylindrical implants that will direct endogenous neural stem cells into regions of interest, such as those damaged by traumatic brain injury or disease.

Disclosures: **L. Hager:** None. **A. Clark:** None. **E. Price:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.18/AA45

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: SENACYT Grant SNI34-2014

Title: Development of a colorimetric assay for the identification of novel compounds as potential therapeutics for Alzheimer's disease

Authors: P. L. FERNANDEZ[1], D. DOENS[1], O. LARIONOV[2], A. TRSITAN[1], A. KHAN[1], *G. B. BRITTON[1], R. LLEONART[1];
[1]INDICASAT AIP, Panama, Panama; [2]Univ. of Texas San Antonio, San Antonio, TX

Abstract: Alzheimer's disease (AD) is the most common form of dementia and represents a major public health problem worldwide. In recent years the potential relevance of microglia innate immune activation in AD has received more attention. Microglia are activated by Amyloid β (Aβ), generating a pro-inflammatory response that plays a critical role in AD pathogenesis. Aβ binds to CD36 and activates microglia to produce cytokines and neurotoxins leading to neurodegeneration. Disruption of this interaction could be relevant to reduce the inflammatory response induced by Aβ. We developed a novel assay to identify molecules able to interfere with the binding of Aβ to CD36. This assay was used to screen libraries of synthetic compounds. Our aims were to: 1) design and optimize an assay to screen for inhibitors of Aβ interaction with CD36, and 2) identify molecules able to impair the pro-inflammatory response mediated by the Aβ-CD36 interaction. For the development of the colorimetric assay we expressed and purified recombinant extracellular domain of the human CD36 protein, which was subsequently used to coat 96 well plates. After incubation with fibrillar Aβ, the complex CD36-Aβ was detected using a mouse anti-Aβ antibody followed by a antimouse HRP-conjugated antibody. The compounds interfering with the binding between CD36 and Aβ were detected by the reduction of the colorimetric signal. Ursolic acid was used as positive control of inhibition. Compounds identified by the assay were tested in a cell-based assay to evaluate their capacity to inhibit the secretion of inflammatory mediators by microglia stimulated *in vitro* with Aβ. The new assay was used for the screening of a total of 128 new synthetic compounds from four different libraries. Twenty five compounds showed inhibitory effect on CD36-Aβ interaction. Preliminary data show that four of those active compounds also have an effect on the microglia response evaluated *in vitro*. These results suggest that this novel assay represents a promising tool for the identification of small molecules targeting inflammation in AD pathogenesis.

Disclosures: P.L. Fernandez: None. **D. Doens:** None. **O. Larionov:** None. **A. Trsitan:** None. **A. Khan:** None. **G.B. Britton:** None. **R. Lleonart:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.19/AA46

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: BBSRC

Title: Exploring methods of cholinergic denervation of the rat ventral tegmental area

Authors: *J. L. FULLERTON;
SIPBS, Strathclyde Univ., Glasgow, United Kingdom

Abstract: Midbrain dopamine (DA) neurons in the ventral tegmental area (VTA) are involved in learning and contribute to the rewarding properties of drugs of abuse. The VTA receives glutamatergic, GABA-ergic and cholinergic inputs. The only known source of cholinergic input to VTA DA neurons is from the mesopontine tegmentum - the pedunculopontine tegmental and laterodorsal tegmental nuclei (PPTg, LDTg). Uniquely in the mesopontine tegmentum, cholinergic neurons express urotensin-II receptors (UII-R). Exploiting this expression, a selective toxin for mesopontine cholinergic neurons was created fusing diphtheria toxin and urotensin-II peptide (Dtx-UII). This toxin destroys cholinergic neurons after direct injection into the PPTg or LDTg but doing either of these only eliminates a proportion of cholinergic VTA input. Is it possible to inject Dtx-UII into the VTA to destroy all cholinergic terminals there? The effect of Dtx-UII on mesopontine cholinergic terminals was assessed through unilateral infusion into the posterior VTA (pVTA) of Dtx-UII (200µL, 3%). To determine whether complete cholinergic denervation of the pVTA could be achieved rats were perfused at various times after surgery (2, 4, 6, 8 & 10 days). Tissue was processed using vesicular acetylcholine transporter (VAChT) to label cholinergic neurons, and fluoro-jade C, which binds to neurodegenerating axons, dendrites and terminals. Control experiments were undertaken infusing ibotenate (180µL, 0.06%) into pVTA. Dtx-UII pVTA infusion did not change VAChT expression 2, 4, 6, 8 & 10 days after surgery. VAChT and fluoro-jade C immunohistochemistry was not altered, which would indicate that cholinergic terminals were not destroyed and that no neurodegeneration had occurred. Tissue infused with ibotenate produced a marked increase in fluoro-jade C positive cells in the pVTA. Further experiments could determine the effect of higher Dtx-UII concentrations: while the dose used here is effective in PPTg, UII-Rs are expressed at a lower level in VTA than PPTg/LDTg. Furthermore, because Dtx-UII is expected to act through retrograde degradation, it may require longer times to initiate cell death. This toxin holds potential to deepen our knowledge of the relationship between the mesopontine tegmental nuclei and VTA, aiding our understanding of how mesopontine cholinergic neurons regulate activity midbrain DA neurons and through them, corticostriatal activity.

Disclosures: J.L. Fullerton: None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.20/AA47

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH

NSF

University of Florida

Title: Real time observation of rodent cerebral metabolism by dissolution dynamic nuclear polarization

Authors: ***D. DOWNES**, B. LAMA, J. COLLINS, M. FEBO, J. LONG;
Univ. of Florida, Gainesville, FL

Abstract: *In vivo* magnetic resonance spectroscopy and imaging (MRSI) is a useful technique for measuring carbon metabolism in live animals, but is often limited because of poor signal-to-noise. Dynamic nuclear polarization (DNP) is a rapidly developing technique in the field of MRS, offering a highly sensitive enhancement to traditional MRSI. Stable isotopically labeled metabolites are hyperpolarized prior to MR spectroscopy allowing for a substantial increase in the carbon signal-to-noise ratio (SNR) of >10,000x. This gain in carbon SNR allows cellular metabolic pathways to be observed in real time. Integration of dissolution dynamic nuclear polarization (dDNP) into MRSI studies of a chronic addiction model is important for investigating changes in neurometabolic processes. Chronic administration of neuro-stimulatory agents has been shown to abate cerebral glucose metabolism in reward regions of the brain. It is our central hypothesis that dDNP can be used to observe this dysfunction in real-time for cocaine addiction and withdrawal. Divergence from normal citric acid cycle metabolism in chronic addiction will be quantified by elevated lactate production in regions of reward in the brain and will provide insight into mitochondrial function as an indicator of addiction associated cerebral damage. We have hyperpolarized metabolites of interest in a 5 T wide-bore dDNP instrument at ~1 Kelvin with a 140 GHz VDI microwave source. Phantom and animal studies have been performed with the use of an automated injection system coupled to a 4.7 T and 11 T MRI instrument.

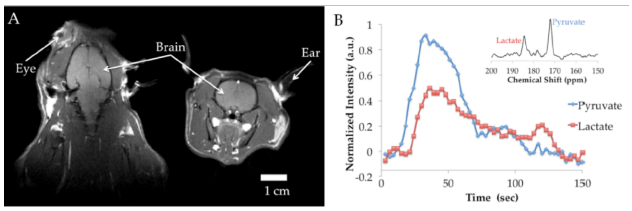

Figure 1. Preliminary cerebral MR images and metabolic spectrum of an untreated wild-type rat. [A] Proton MRI axial and coronal slice image of a normal rat brain at 4.7 T. [B] Time resolved hyperpolarized pyruvate and lactate product brain metabolism with average carbon spectra and assigned peaks (inset).

Disclosures: **D. Downes:** None. **B. Lama:** None. **J. Collins:** None. **M. Febo:** None. **J. Long:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.21/AA48

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Adenosine as a parameter to determine the quality of the rapid microwave fixation of rat brain for the determination of cAMP and cGMP in braintissue

Authors: A. RASSOULPOUR, F. HELFRICH, *M. G. VAN DER HART;
Brains On-Line, South San Francisco, CA

Abstract: Cyclic adenosine-3',5'-monophosphate (cAMP) and cyclic guanosine-3',5'-monophosphate (cGMP) are important second messengers for intracellular communication. Illnesses and disorders that affect the brain can show an impaired intracellular communication. Quantification of these metabolites can elucidate regulation and dysregulation of these important processes, and serve as biomarkers for therapeutics that target cAMP/cGMP regulation Measuring cAMP and cGMP in brain tissue can be a challenging due to their rapid degradation by enzymes (i.e. phosphodiesterase's), which are still active postmortem. Using rapid microwave fixation, the enzymes are instantly deactivated and thus prevents the degradation. Unfortunately it is hard to determine the quality of the fixation. In this study we investigated if adenosine, an end product of cAMP degradation, could be used as a reliable parameter to quantify the successfulness of brain fixation. For this purpose we performed rapid microwave fixation followed by brain dissection. The prefrontal cortex, striatum and ventral hippocampus were collected and quickly frozen and stored until analysis. Tissues were homogenized in perchloric acid and assayed by LC-MS/MS for cAMP, cGMP and adenosine. It was found that concurrent measurement of adenosine was critical in determining sample integrity for cAMP and cGMP analysis by indicating which samples were not properly fixed. It was found that brain areas furthest away from the center of the beam had more often a significantly higher adenosine concentration than the brain areas located in the center of the microwave beam during the rapid

fixation process. Relative to samples with normal adonesine levels, samples displaying high adonesine levels had lower levels of cAMP concentrations at 64%(n=6) in the cortex, 60%(n=19) in hippocampus, 58%(n=16) in striatum and cGMP concentrations at 54%(n=6) in the cortex, 46%(n=15) in hippocampus, and 50%(n=14) in striatum. To test if this method could reliably measure differences in cAMP and cGMP after pharmacological manipulations animals were treated with Rolipram, a non-specific phosphodiesterase inhibitor. This yielded cAMP cortex concentrations higher than vehicle treated animals, while no significant difference was observed with respect to cGMP concentration in the brain.

Disclosures: A. Rassoulpour: None. **F. Helfrich:** None. **M.G. van der Hart:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.22/BB1

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH NS059622

NIH NS050243

Title: A rapid method of isolating purified population of Schwann cells for spinal cord transplantation

Authors: *W. WU, W. QU, X.-M. XU;
Neurolog. Surgery, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Accumulated evidence suggests that Schwann cell (SC) transplantation is one of the most promising cell therapies for repair after acute and chronic spinal cord injuries (SCIs). For clinical application, transplantation of autologous SCs is preferred to avoid immune rejection of grafted cells. Conventional SC culture method requires lengthy *in vitro* procedures to generate large quantity of purified SCs, which may miss the optimal therapeutic window following an acute SCI. Thus, obtaining large quantities of highly purified and viable SCs within a short period of time is very important in both experimental and translational SCI research. In 2012, Kaewkhaw et al. developed a new protocol utilizing customized DMEM depending on the preferential expression of D-amino acid oxidase (DAAO) in SCs (Kaewkhaw, R., et al. Nat. Protocol 7: 1996-2004, 2012). This method eliminated the fibroblast purifying procedure as required in other methods, and showed a great potential for rapidly isolating high purity SCs.

However, the biological properties of SCs isolated with this method remains unclear. In this study, we used green fluorescence protein (GFP) transgenic SD rats as the donor for isolating SCs. To exclude the possibility that the GFP gene may affect the property of SCs, we firstly used a traditional method to isolate SCs using sciatic nerve explant culture (Xu et al. J Comp. Neurol. 351:145-160, 1995) and compared SC cultures isolated from GFP transgenic (GFP-SC) and wide type SD rats (WT-SC). The result showed no significance difference between isolated GFP-SCs and WT-SCs in terms of purity and proliferation. We then cultured GFP-SCs using the freshly dissociate method of Kaewkhaw and compared them with the GFP-SCs cultured with the traditional method in terms of purity and proliferation. The result showed that SCs isolated from freshly dissociated adult sciatic nerves achieved a high purity (>99%) in a shorter culture period (19 days), as compare to the traditional method (44-48 days). At day 16, SCs were transferred from 3.5 mm dishes to a T25 flask and, at day 18, to a T75 flask. Using the new method, it took only 2 days for SCs to become confluent in a T75 flask, while it took at least 3-4 days when a traditional method was used, indicating that SCs proliferated faster with the new culture protocol. To approve the functionality of SCs isolated with the new method, an ongoing study is being conducted to determine the survival, migration , axon growth promotion, and myelination of grafted SCs in a rat spinal cord contusion injury model at the 10th thoracic level (T10).

Disclosures: **W. Wu:** None. **W. Qu:** None. **X. Xu:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.23/BB2

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH grant 1R01AG042890 to GT

Title: A method to determine *ex vivo* insulin responsiveness in synaptosomes isolated from frozen brain tissue

Authors: *W. FRANKLIN[1], G. TAGLIALATELA[2];
[2]Neurol., [1]UTMB At Galveston, Galveston, TX

Abstract: Alterations of insulin signaling in neurons have been linked to many disorders such as diabetes, inflammation, and Alzheimer's disease (AD). Synapses are rich of insulin receptors and insulin has been shown to be important to maintain synaptic health/integrity. Notably, decreased insulin signaling (insulin resistance) increases synaptic sensitivity to amyloid beta (Aβ), the toxic

protein that accumulates in AD, thus contributing to the cognitive decline that characterizes this neurodegenerative disorder. Therefore, studying the insulin signaling response at the synapse is an important approach to understand molecular mechanisms involved in disease-related neurodegenerative processes and test the effectiveness of potential new treatments. With this goal in mind, we have developed a method for studying the insulin responsiveness at the synaptic level by isolating functional synaptosomes from tissue and exposing them to insulin in the presence of ATP to detect receptor and associated pathway activation. Using this method coupled to Western blot analysis, we were able to detect insulin-driven phosphorylation of the insulin receptor (IR) as well as phosphorylation of other signaling elements in the insulin pathway including IRS1, AKT, and GSK3β. Furthermore, effective phosphorylation of the IR in isolated synaptosomes could be observed using either insulin or IGF-1, further indicating the presence of a functional IR that responds to multiple ligands as it naturally does in the CNS. We optimized this method by performing dose response curves of insulin and ATP as well as an insulin time course on IR activation and obtained reliable results using synaptosomes isolated from both fresh and frozen tissue from rats and mice. We have also examined the effect on the response of the insulin receptor, AKT, and GSK3β of storing isolated synaptosomes frozen for extended periods of time prior to insulin stimulation as well as determining the impact of varying post-mortem intervals prior to tissue collection. Our results showed differential impact on different elements of the insulin signaling pathway depending on varying experimental conditions, indicating the need for maintaining a tightly standardized approach across different experiments. In conclusion, we have developed a reliable method to measure *ex vivo* phosphorylation of the insulin receptor and associated signaling pathway. To the best of our knowledge, this is the first evidence of stimulation of isolated synaptosomes with insulin and a promising new technique to study the synaptic CNS insulin responsiveness under disease conditions.

Disclosures: W. Franklin: None. **G. Taglialatela:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.24/BB3

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: HHMI Investigator Funds

Title: Technologies for targeting and manipulating cells based on intracellular products

Authors: *C. TANG[1], S. RUDOLPH[1], T. SZIKRA[2], E. DROKHLYANSKY[1], G. KOZOROVITSKIY[3], M. TEXEIRA[2], O. DHANDE[4], V. E. ABRAIRA[1], S. CHOI[1], S. WANG[1], B. GUO[1], S. LAPAN[1], I. R. DREW[1], B. L. SABATINI[6], A. HUBERMAN[5], B. ROSKA[2], W. G. REGEHR[1], C. L. CEPKO[6];
[1]Harvard Med. Sch., Boston, MA; [2]Friedrich Miescher Inst. for Biomed. Res., Basel, Switzerland; [3]Northwestern Univ., Chicago, IL; [4]Univ. of California San Diego, San Diego, CA; [5]Univ. of California San Diego, San Diego, MA; [6]Harvard Med. School, HHMI, Boston, MA

Abstract: Studies of complex multicellular organisms would benefit from the ability to selectively manipulate the activities of any cell type of interest. Our ability to achieve this is currently limited by technology and available resources. Here, I explore the artificial use of intracellular proteins as signals for conferring cell specificity in gene manipulation. The Green Fluorescent Protein (GFP) is a useful marker of gene expression and thousands of transgenic GFP reporter lines have been made to label different cell types, particularly in the mouse nervous system. However, the utility of transgenic GFP reporter lines is limited to labeling purposes. I exploited this resource for cell-specific gene manipulation by constructing synthetic systems that become biologically active upon interaction with GFP. Using GFP-binding nanobodies derived from Camelid antibodies, I co-opted GFP as a scaffold protein to bring together complementary split proteins that, when in a complex, can regulate processes such as transcription and DNA recombination. I demonstrate the utility of these systems for selectively manipulating GFP-expressing cells in the mouse nervous system and in zebrafish, for applications such as developmental perturbations, electrophysiology and optogenetic interrogation of neural circuits. In addition to the above, I will discuss current efforts to simplify the complexity of GFP-dependent systems. Thus, my work expands the experimental paradigm for manipulation of specific cell types in multicellular organisms and provides novel tools and approaches for cellular-level analysis of biological processes in animals.

Disclosures: C. Tang: None. **S. Rudolph:** None. **T. Szikra:** None. **E. Drokhlyansky:** None. **G. Kozorovitskiy:** None. **M. Texeira:** None. **O. Dhande:** None. **V.E. Abraira:** None. **S. Choi:** None. **S. Wang:** None. **B. Guo:** None. **S. Lapan:** None. **I.R. Drew:** None. **B.L. Sabatini:** None. **A. Huberman:** None. **B. Roska:** None. **W.G. Regehr:** None. **C.L. Cepko:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.25/BB4

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: CIHR Grant MOP-111220

Title: Two-photon optogenetics with near-infrared light-activated cyclases and FRET sensors for studying the role of cAMP and cGMP in living neurons

Authors: *M. VALENCIA[1,2], T. T. LUYBEN[1,2], K. OKAMOTO[1,2];
[1]Lunenfeld-Tanenbaum Res. Inst., Mount Sinai Hosp., Toronto, ON, Canada; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Optogenetics provides powerful tools to non-invasively manipulate neural activity and specific protein function by light. However, for studying intracellular signaling mechanisms involved in synaptic function, these optogenetic tools are not yet well established. Here, we describe two-photon optogenetics with near-infrared light-activated cyclases and FRET sensors for elucidating the roles of intracellular messengers cAMP and cGMP in the synapses of living neurons. To dissect the role of cAMP in synaptic plasticity, we have utilized a bacterial photoactivatable adenylate cyclase (PAC) which produces cAMP in response to blue light. We demonstrated photoactivation of PAC at the single synapse level by two-photon excitation light in cultured hippocampal slices. To expand our two-photon optogenetic approach for studying intracellular messengers, we optimized and established a near-infrared light-sensitive adenylate cyclase (NIR-PAC), a fusion of a photosensory PAS-GAF-PHY domain with a catalytic domain of adenylate cyclase (Ryu *et al.*, 2014), for two-photon photoactivation. Also, by mutating the enzymatic pocket of NIR-PAC, we engineered a near-infrared light-sensitive guanylate cyclase (NIR-PGC). To confirm light-dependent activation of these near-infrared light-activated cyclases, we excited each cyclase with LED light (660 nm peak) *in vitro* and measured cAMP or cGMP levels by ELISA. The intracellular messenger levels were raised within seconds after light stimulation, indicating rapid photoactivation. We then examined enzymatic activity across the two-photon excitation spectra *in vitro* to optimize the excitation wavelength. Two-photon excitation wavelengths greater than 1000 nm efficiently activated both NIR-PAC and NIR-PGC, suggesting the ability to control their activation at single synapses using two-photon microscopy. Since we have found that the two-photon excitation range of the blue-light sensitive PAC is up to 1000 nm, the two-photon excitation range of the near-infrared cyclases suggests the possibility to manipulate cAMP and cGMP levels separately using different wavelengths of two-photon excitation light in the same living neurons. By optimizing genetically-encoded two-photon FRET (Förster Resonance Energy Transfer) sensors for cAMP and cGMP, we validated the near-infrared light-sensitive cyclases in living pyramidal neurons of hippocampal slice cultures. We will discuss how these two-photon optogenetic technologies will serve as valuable tools for elucidating the role of postsynaptic cAMP and cGMP signalling cascades in living neurons.

Disclosures: **M. Valencia:** None. **T.T. Luyben:** None. **K. Okamoto:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.26/BB5

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: CIHR: MOP-111220

Title: Two-photon optogenetics for controlling the activity of PDEs in living neurons

Authors: *F. BERGIN, K. OKAMOTO;
Samuel Lunenfeld Res. Inst., Toronto, ON, Canada

Abstract: Phosphodiesterases (PDEs) catalyze the hydrolysis of intracellular second messengers, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), which are essential for synaptic plasticity underlying learning and memory. PDEs have been targeted for drug discovery in a variety of psychiatric and neurodegenerative disorders including schizophrenia and Alzheimer's disease. However, the exact function of PDEs in synaptic plasticity is still unclear due to the lack of techniques to locally monitor and control their activity in the brain. Current pharmacological and genetic approaches for studying PDEs are limited by cellular precision and spatiotemporal specificity. Here we report the two-photon optogenetic tools to manipulate PDEs activity by light in a spatiotemporally precise manner, to study their synaptic function. Mammalian PDEs are divided into 3 types: cAMP specific (PDE4, 7, 8), cGMP specific (PDE5, 6, 9) and PDEs that hydrolyze both (PDE1-3, 10, 11). All types of PDEs are expressed in central nervous system such as hippocampus, cortex and striatum, suggesting their distinct roles for synaptic function. To establish photoactivatable PDEs, we utilized a recently described optogenetic strategy fusing a far-red light sensitive domain PAS-GAF-PHY to the catalytic domain of PDE2A (Moglich *et al*, 2014). We prepared a series of variants and optimized the 3 types of photoactivatable PDEs for PDE2, 4 and 5 *in vitro*. The light activation of photoactivatable PDE2 indicated the hydrolysis of applied cAMP and cGMP within seconds by light, indicating its strong photoactivation. In contrast photoactivatable PDE4 and 5 showed their photoactivation of hydrolysis only for cAMP (PDE4) or cGMP (PDE5), demonstrating their target specificity. Next, to study the synaptic level function in the mouse brain, we use two-photon microscopy to achieve activation of photoactivatable PDEs at the single synapse level in living tissues. To optimize the two-photon excitation light-dependent activity of each enzyme, we examined the excitation spectra of photoactivatable PDEs *in vitro*. We found the longer two-photon excitation wavelengths corresponding to the single photon excitation range of the far-red light sensitive domain efficiently activated these PDEs *in vitro*, suggesting the ability to control

its activation at single synapses. We will discuss their use in *in vivo* studies using hippocampal slice cultured neurons.

Disclosures: F. Bergin: None. **K. Okamoto:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.27/BB6

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: JST PRESTO

JSPS Grant-in-Aid for Young Scientists (B) 21700412

MEXT Strategic Research Program for Brain Sciences

Title: Optical inactivation technology of synaptic AMPA receptors *in vivo*

Authors: *K. TAKEMOTO[1], H. IWANARI[2], T. NAGAI[3], T. HAMAKUBO[2], T. TAKAHASHI[1];
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Abstract: Acute inactivation of synaptic neurotransmitter receptors *in vivo* with spatio-temporal precision should be a powerful tool to understand their roles in physiological functions. Among many molecules in synapse, AMPA type glutamate receptor, GluA1 is an especially known as important molecules for memory acquisition which is delivered into synapses in response to many types of learning and experience. Here, we developed an optical technology for acute inactivation of synaptic GluA1 receptors by CALI (chromophore-assisted-light-inactivation) *in vivo*. CALI uses a photosensitizer which produces short-lived reactive oxygen species, such as singlet oxygen, by irradiation with light. Singlet oxygen shows a half-radius of photodamage for approximately 3-4nm, which is smaller than the average protein-protein interaction distance (8nm) (Linden et al., 1992; Beck et al., 2002). Thus, CALI could promise the specific inactivation of a target protein with high spatial precision which is difficult for gene knockout and RNAi. Further, by using antibodies labelled with photosensitizer, we could inactivate a protein on the cell surface without damaging neighboring proteins. To develop the anti-GluA1 antibody fo CALI experiments, we raised and screened a monoclonal antibody against the extracellular domain of GluA1 to induce specific CALI by labeling with photosensitizer, eosin

(Takemoto et al 2011). In response to green light, one labeled-antibody effectively and specifically inactivated GluA1 receptors both in recombinant AMPA receptor expressing cells and endogenous AMPA receptor expressing neuronal synapses. Using this system, we will also talk about a novel *in vivo* optical technology for artificial memory erasure by *in vivo* CALI system. Our optical technology will permit their physiological roles in memory acquisition and formation to be analyzed with high spatio-temporal resolution, such as individual spines, and ultimately enable us to map out "the functional synapses" in memory system.

Disclosures: K. Takemoto: None. **H. Iwanari:** None. **T. Nagai:** None. **T. Hamakubo:** None. **T. Takahashi:** None.

Poster

091. Biochemical Techniques

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 91.28/BB7

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: The Grainger Foundation

NIH Grant U01 NS090455

NIH Grant R01 NS075013

Title: Development of boron-doped diamond microelectrodes for human use: electrode engineering and fabrication

Authors: *J. TOMSHINE[1], K. BENNET[1], F. MANCIU[2], M. MARSH[1], M. SETTELL[1], E. NICOLAI[1], K. LEE[1];
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Abstract: Introduction: Deep Brain Stimulation (DBS) is a neurosurgical intervention that has attracted widespread interest in recent years, yet the technique remains limited by its "open-loop" nature. That is, modern DBS implants do not automatically adjust their level of stimulation based on any measured physiological parameter, thus creating a potential for either over- or under-treatment. Fast scan cyclic voltammetry (FSCV) is a well-established analytical technique that is able to measure - *in vivo* - the neurotransmitters that are released as a result of DBS. However, existing electrode designs for FSCV rely largely on carbon fiber and degrade quickly at peak potentials above 1.2-1.4 V. This makes existing electrodes unsuitable for chronic implantation if neurotransmitters such as adenosine (whose oxidation potential occurs above this range) are to

be measured. This work presents the design and fabrication of a durable synthetic diamond-based electrode capable of measuring physiologically-relevant levels of neurotransmitter for far longer than prior carbon fiber-based electrodes. Methods: Hot-filament chemical vapor deposition was used to deposit boron-doped polycrystalline diamond coatings on sharpened tungsten substrates. The purity of the diamond coatings was evaluated by Raman spectroscopy. Longevity was evaluated *in vitro* by continuous application of the FSCV waveform over several days in buffer solution with periodic calibration using dopamine. Electron microscopy was used to image electrode tip degradation. Results: The diamond electrodes constructed in this work retained their sensitivity over a timespan that caused conventional carbon fiber electrodes to fail (5.2 million FSCV measurement cycles). The carbon fiber electrodes were physically degraded, while diamond electrodes remained unchanged (and sensitive to dopamine) following equivalent treatment. The diamond electrode tips also require more than 2 orders of magnitude more physical force to break - an important characteristic for long-term surgical implantation. Conclusion: The diamond electrodes presented here, coupled with fast scan cyclic voltammetry, provide a promising sensing platform for the design of a closed-loop deep brain stimulation device. While the electrodes used in this study were not packaged for chronic implantation, they maintained sensitivity for a number of cycles sufficient to make chronic implantation practical.

Disclosures: **J. Tomshine:** None. **K. Bennet:** A. Employment/Salary (full or part-time):; Mayo Clinic. **F. Manciu:** None. **M. Marsh:** None. **M. Settell:** A. Employment/Salary (full or part-time):; Mayo Clinic. **E. Nicolai:** None. **K. Lee:** None.

Poster

091. Biochemical Techniques

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH (R01 Ns75013)

Grainger Foundation

Title: Carbon nanofiber microelectrode needle array fabrication and characterization

Authors: ***M. P. MARSH**[1], J. E. KOEHNE[5], C. KIMBLE[2], S.-Y. CHANG[1], P. MIN[3], R. ANDREWS[5], M. MEYYAPPAN[5], K. E. BENNET[4], K. H. LEE[3];
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Abstract: Background: Electrochemical methods for neuroscience research have traditionally employed a single carbon fiber, aspirated into a glass capillary, as an electrode. While this design has proven successful for detection of neurotransmitters *in vivo* and *in vitro*, it has some drawbacks: It is very fragile, and it can record neural activity only in a single small region of the brain. Here we describe the fabrication and characterization of a penetrating silicon-substrate based multiplex microelectrode array that utilizes aggregations of carbon nanofibers (CNF) as sensing elements. Methods: Device fabrication was performed on a silicon-on-insulator wafer using the following main steps: (1) metal conductor deposition; (2) Ni catalyst deposition; (3) CNF growth by plasma enhanced chemical vapor deposition; (4) CNF encapsulation with dielectric; (5) 1 μm removal of top dielectric layer to expose CNF tips and (6) deep reactive ion etch (DRIE) for electrode release from the base wafer. The resulting electrode design is 5 mm in length, 50 μm thick, and 120 μm at the widest point, with six sensor pads and approximately 40,000 nanofibers per sensor. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) were performed to characterize the surface structure of the carbon nanofiber sensing elements. The electrode was then interfaced with a printed circuit board via adhesive and wire-bonding, and the connections were electrically insulated with a light-activated polymer resin. Subsequent *in vitro* electrochemical characterization was performed in a flow cell using the Mayo-developed WINCS Harmoni to perform fast-scan cyclic voltammetry (FSCV). Results: The fabrication steps highlighted above produced a functional multiplex electrode array containing 6 sensing pads per device. CNF growth and exposure was confirmed with SEM and AFM techniques. FSCV demonstrated the electrode was sensitive to both dopamine (DA) and oxygen (O2). Additionally, simultaneous detection of mixtures of DA and O2 on adjacent electrode pads with minimal crosstalk was demonstrated with the WINCS Harmoni system. Conclusion: Due to the advantages of multiplexing and simultaneous detection of multiple analytes afforded by the CNF microelectrode array, we believe that the initial testing of silicon-based CNF electrodes shows promise for future applications in the electrochemical detection of neurotransmitters, e.g. dopamine, serotonin, etc. Potential applications include elucidating the mechanisms of action of deep brain stimulation (DBS) and intraoperative neurotransmitter monitoring during neurosurgery. Funding: Support by NIH (R01 Ns75013) and the Grainger Foundation.

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Poster

092. Bioinformatics

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Program#/Poster#: 92.01/BB9

Topic: G.06. Computation, Modeling, and Simulation

Support: ANR-14-NEUC-0003

Title: Mechanistic modeling of spike-timing dependent plasticity of basal ganglia neurons

Authors: I. PROKIN[1], S. VALTCHEVA[2], L. VENANCE[2], *H. BERRY[1];
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Abstract: Synaptic plasticity is a plausible support for learning and memory in single neurons. A recent plasticity paradigm is spike-timing dependent plasticity (STDP), whereby synaptic weight change is dictated by the relative timing of paired pre- and postsynaptic action potentials (AP). Notwithstanding the large volume of research on STDP, its dependence on underlying signaling pathways is not fully understood. To address this issue, we combined electrophysiology experiments with modeling of the implicated signaling network comprising the NMDAR and endocannabinoid (eCB) pathways. We have elaborated the model using experimental data obtained with typical STDP protocols (100 AP pairings at 1 Hz) at corticostriatal synapses. STDP at these synapses is known to be anti-Hebbian: long-term depression, LTD, is induced when the presynaptic AP occurs before the postsynaptic one (pre→post) whereas long-term potentiation, LTP, is induced by the reverse order (post→pre). The model reproduces this main feature. It was then validated against additional data consisting mostly in varying the number of AP pairings and manipulating astrocytic glutamate uptake (see the related experimental poster by Valtcheva and Venance). The model again reproduces all key experimental results considered. Together, confrontation with the experiments yielded the following insights: (i) the contribution of NMDAR and eCB pathway to STDP vary with the number of AP pairings: while NMDAR-dependent LTP and eCB-dependent LTD need large numbers of pairings, eCB-dependent LTP is expressed only with few pairings (5 to 25); (ii) eCB-dependent plasticity is thus bidirectional: its outcome (LTD or LTP) depends on the amount of eCBs produced during the protocol; (iii) STDP is controlled by glutamate uptake by the astrocytic glutamate transporter EAAT2 (GLT-1). This joint experimental-modeling approach has thus proven useful for the study of the molecular basis of synaptic plasticity; its further development might significantly advance our understanding of synaptic plasticity in normal and pathological conditions.

Disclosures: I. Prokin: None. **S. Valtcheva:** None. **L. Venance:** None. **H. Berry:** None.

Poster

092. Bioinformatics

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Topic: G.05. Bioinformatics

Support: Indian Council of Medical Research, New Delhi

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NIH Grant AG047812

Title: A systematic approach to analyze the impact of p53 mutations on p53-mdm2 interaction using machine learning

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Abstract: BACKGROUND: p53 protein is known to play a critical role in mediating the several cellular mechanisms, including as cell cycle arrest, apoptosis, and cellular senescence. Its functions are largely implicated in several neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Most importantly, the oncoprotein MDM2 is known to bind to the N-terminal transactivation domain of p53, which promotes tumorigenesis. The purpose of our study was to understand the physiological relevance of p53 and MDM2 interactions in tumors. METHODS: Machine learning has been used in several applications of biomedical research where for a given datasets an acceptable generalization will be obtained using different algorithms. In this study, a diverse predictive model was employed to perform predictions on the impact of mutations in p53-MDM2 interaction. p53 mutations were collected from the public domain databases and literature search using Pubmed. Molecular interactions between the complexes were analyzed using protein-protein docking servers. Mutations in the p53 transactivation domain were induced using pymol followed by the energy minimization. Predictive models for the mutant and native complexes were built using artificial neural network algorithms. RESUTS: Results showed that a large deviation in the total solvent accessible surface area, structure solvent energy and average gain in the complex formation properties of mutant p53-MDM2 complexes compared to the native ones. Neural network analysis showed that structural features are important for predicting the impact of mutants on p53-MDM2 interaction. CONCLUSIONS: Based on the analysis of results, it is evident that the integration of machine learning approaches can provide a promising inference on the impact of mutations on the protein-protein interactions

Disclosures: **S. Yeguvapalli:** None. **K. Chitrala:** None. **P. H. Reddy:** None.

Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH Grant 1R01EB014641-01

Title: Model-based control of spreading depression

Authors: ***S. VAN WERT**[1], S. J. SCHIFF[2];
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Abstract: Spreading depression is a wave-like neural phenomenon that occurs in the cortex of the human brain and is linked to disorders of the brain such as migraine, epilepsy, and stroke. We propose a cellular model of the brain that, with only differences in environmental conditions, replicates the cellular dynamics of these varying pathological phenomena, and with this model we create a model-based control scheme that could be used in novel treatment technologies for those pathologies in a clinical setting. The model's capability of unifying seemingly different pathological phenomena with the same set of modeled cellular dynamics is created by a few important changes to standard neural modeling. Notably among those changes are the tracking of the large deviations in the ionic concentrations of the cellular environment and also the tracking of the energy use and volume regulation of the cell as it responds to disruptions from homeostasis. These properties are critical for modeling of pathological dynamics like SD, but are typically ignored in standard computational neural models of healthy tissue. Building and investigating a model of spreading depression that tracks these variations allows us to determine best practices for understanding and treating or preventing the disorders it is associated with in real brain tissue. To demonstrate the possible use of this model in translational and clinical applications, we describe a model-based control mechanism for interacting with the cellular environment by application of electrical field as a route to possible treatment. Global application of electric field can both affect the transport of ions along diffusion channels that buffer extracellular ion concentrations space, and an electric field can also affect current running both along and across the membrane in a spatially extended neuron. In search of viable control schemes for potential use in treatment, we demonstrate the plausibility and effectiveness of several model-based control schemes to alter the neural dynamics so as to prevent or modulate

the pathological phenomenon of not only SD but related pathophysiological behaviors of seizure and stroke as well. The implemented controllers provide novel approaches for universal treatment of pathologies in neural tissue. These treatments would maintain a stable environment that avoids excessive demands on energy, as well as significant disruptions to the local ionic environment. This model-based control lays a foundation for medical technology that monitors critical features of brain dynamics and supplies an appropriate and safe treatment stimulus.

Disclosures: S. Van Wert: None. **S.J. Schiff:** None.

Poster

092. Bioinformatics

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Program#/Poster#: 92.04/BB12

Topic: G.05. Bioinformatics

Support: R43MH100780

Title: Validation of a novel 3D particle tracking tool for next generation neuroscience microscopy

Authors: *S. V. ALWORTH[1], M. J. JONES[1], V. CHOU[2], D. VAN VACTOR[2], J. S. J. LEE[1]; [1]DRVision Technologies LLC, Bellevue, WA; [2]Dept. of Cell Biol., Harvard Med. Sch., Boston, MA

Abstract: Time-lapse, 3D imaging of functional neural networks, composed of many neurons connected through a complex web of synapses, is a promising approach for gaining in-depth understanding of how the central nervous system (CNS) works. Using high speed confocal fluorescence microscopy, 3D image sequences are routinely acquired to elucidate the development of functional circuits, as well as the molecular kinetics and interactions that drive CNS development or pathological degeneration. It is now possible to image more complex and intact neural circuits in the CNS *in situ* with high lateral and axial resolution. Imaging of these new model systems could unleash a new generation of scientific inquiries that would lead to new discoveries and therapies. However, 3D neuronal image sequences exhibit low signal to noise ratio (SNR) while the more physiological environment exacerbates the complexity of molecular particle motion. Therefore, quantification of particle dynamics and molecular interactions in these complex models is challenging due to the lack of adequate 3D image analysis methods. To meet the above challenges, we have developed a novel 3D particle tracking method and software prototype, designed for highly sensitive particle detection in low SNR images, and also for

adaptive tracking of particles with heterogeneous types of motion. Adaptive tracking is enabled through a general computational framework that represents object states and state transitions dynamically, and provides for state dependent feature point detection and track matching. In addition, two methods of track matching error correction are employed; one that uses motion energy independent of matching and other that uses user defined state profiles to guard against invalid state transitions. In this study, we use simulated images containing heterogeneous motion, variable particle densities and noise levels, and non-simulated biological images to validate the new methods. We quantitatively evaluate the robustness of the new method to different noise levels and particle densities, and benchmark against common particle tracking methods. Lastly, we compare to manual analysis on real, non-simulation 3D image sequences. Our objectives are that our new 3D tracking tool should 1) perform at least as well as our 2D tracking algorithms benchmarked previously using stand metrics from the IEEE particle tracking challenge (α, β, and the Jaccard similarity index for positions and tracks), and 2) that there should be no significant difference from the manual analysis on real images. We conclude that the new tool meets the objectives and compares well against conventional methods of 3D particle tracking.

Disclosures: **S.V. Alworth:** A. Employment/Salary (full or part-time):; DRVision Technologies LLC. **M.J. Jones:** A. Employment/Salary (full or part-time):; DRVision Technologies LLC. **V. Chou:** Other; DRVision Technologies LLC via NIMH. **D. Van Vactor:** Other; DRVision Technologies LLC via NIMH. **J.S.J. Lee:** A. Employment/Salary (full or part-time):; DRVision Technologies LLC.

Poster

092. Bioinformatics

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 92.05/BB13

Topic: G.06. Computation, Modeling, and Simulation

Title: Neuronal network bump attractors augmented by calcium up-regulation of Ih in a multiscale computer model of prefrontal cortex

Authors: ***A. SEIDENSTEIN**[1,2], S. A. NEYMOTIN[3], A. FESHARAKI[3], M. L. HINES[4], R. A. MCDOUGAL[4], A. S. BULANOVA[3], W. W. LYTTON[3];
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Abstract: Prefrontal cortex (PFC) is thought to be a site of short term memory based on persistent neuronal activity. Previous models have focused on the role of network connectivity via excitatory interconnections in maintaining activation to represent memory through bump attractors. We extended this model by taking account of roles of subcellular and cellular dynamics. A multiscale NEURON model was used to study dynamics across molecular, cellular and network scales. We focused on the role of calcium influence, via calcium-induced calcium release from endoplasmic reticulum (ER), on the hyperpolarization-activated cyclic-nucleotide gated (HCN) channels that produce Ih. This release was influenced by the network through the influence of metabotropic glutamate receptors (mGLUR) in excitatory neuron dendrites which produced IP3 through a G-protein mediation. The network itself was represented by 776 multicompartmental excitatory and inhibitory neurons arranged in the canonical cortical layers, and connected via metabotropic and ionotropic receptors (AMPA/NMDA, GABAA/GABAB). The network enabled individual neurons to maintain tonic NMDA synaptic activation to maintain sustained firing via mutual activation after a strong, brief excitatory stimulation, the classical bump attractor model of Wang et al (J Neurosci 19: 9587,1999). Activity was further augmented through cellular mechanisms, with higher availability of Ca up-regulating Ih, given preceding inhibitory-activation of Ih in the individual neuron through tonic bombardment of GABAA. GABAA therefore played a dual role -- preparing a population for greater augmentation through activation of Ih and also suppressing non-selected cells during the period of bump activation. Glutamatergic activation also played a dual role: metabotropically preparing cells by allowing higher ER storage of Ca prior to stimulation, and ionotropically maintaining activation during the bump. The interactions between the metabotropic and ionotropic inputs to the neuron demonstrated how multiple pathways could contribute in a complementary manner to persistent activity. The network showed a complex interdependence between synaptic weights, excitation/inhibition balance, firing rates, membrane depolarization, Ca levels, regulation of HCN, and induction of persistent activity. The ability to mediate activation at different time scales, and through different pathways, would be expected to protect against disruption, in this case providing stability for working memory.

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Poster

092. Bioinformatics

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Topic: G.06. Computation, Modeling, and Simulation

Support: Brain Korea 21 PLUS Project for Medical Science, Yonsei University

Yonsei-SNU Collaborative Research Fund of 2014

Title: Enhanced neuronal growth by intracellular stimulation

Authors: *H. LEE[1], I. KIM[2], Y. HA[1], H.-J. CHOI[2], S. YI[1];
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Abstract: Electrical stimulation through direct electrical activation has been widely used to recover the function of neurons, primarily through the extracellular application of thin film electrodes. However, studies using extracellular methods show limited ability to reveal correlations between the cells and the electrical stimulation due to interference from external sources such as membrane capacitance and culture medium. Here, we demonstrate long-term intracellular electrical stimulation of undamaged pheochromocytoma (PC-12) cells by utilizing a vertical nanowire electrode array (VNEA). The VNEA was prepared by synthesizing silicon nanowires (SiNWs) on a Si substrate through a vapor-liquid-solid (VLS) mechanism, and then fabricating them into electrodes with semiconductor nanodevice processing. PC-12 cells were cultured on the VNEA for 4 days with intracellular electrical stimulation, and then a 2-day stabilization period. Periodic scanning via two-photon microscopy confirmed that the electrodes pierced the cells without inducing damage. Electrical stimulation through the VNEA enhances neuronal differentiation and neurite outgrowth by about 50% relative to extracellular stimulation under the same conditions. VNEA-mediated stimulation also revealed that cellular differentiation and growth in the cultures were dependent on the potential used to stimulate them. Intracellular stimulation using nanowires could pave the way for controlled neuronal differentiation and outgrowth studies in living cells.

Disclosures: **H. Lee:** None. **I. Kim:** None. **Y. Ha:** None. **H. Choi:** None. **S. Yi:** None.

Poster

092. Bioinformatics

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Topic: G.05. Bioinformatics

Support: UK EPSRC EP/E002331/1

UK BBSRC BB/IO01042/1

Title: Sharing electrophysiological data and the CARMEN project

Authors: *L. S. SMITH[1], E. SERNAGOR[2];
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Abstract: Many journals and research funders now demand that researchers make their datasets, metadata, and processing software publically and permanently available once their work is published: indeed, repeatability of results and re-use of materials for cross-analysis requires this. Often the mechanism for archiving is unspecified. The International Neuroinformatics Co-ordinating Forum (INCF) keeps a list of re-usable Neuroscience (including Neurophysiology) resources at http://incf.org/resources/research-tools. In addition, the INCF's Electrophysiology Task Force maintains a list of resources for data sharing in (Neuroimaging and) Electrophysiology at http://tinyurl.com/d7f35qb. However, many of these are intended for specimen datasets, rather than data generated during research. The UK based CARMEN project (https://portal.carmen.org.uk) is a portal based collaborative facility for neuroscientists (and in particular electrophysiologists) to share data and tools for working on data. Started 2006, it has been providing a gradually improving service for about seven years. The system can translate many common formats into has its own internal format: it now also has the ability to generate a quick graphical display of datasets. Though successful for enabling geographically distributed groups to share data and techniques, it has been less successful at being used for publically available data. Neuroinformaticians are already convinced of the importance of data sharing, so it is imperative to find out what is impeding uptake by experimentalists. Following a questionnaire issued to users (and non-users) we found that the issues were partly ease of uploading (speed, and issues with metadata), difficulties in finding and using services and developing and using workflows. Many users only wanted archival capability, and found the system complex. Yet other users have used the system in a much more powerful way, performing historic cross-analyses which hopefully will pave the way for others to start using the resource to its full potential. Long term storage and archiving of data and processing techniques demands long term funding of infrastructure projects. Yet although publishers and funders want this, it remains difficult to fund such projects for the long term.

Disclosures: L.S. Smith: None. **E. Sernagor:** None.

Poster

092. Bioinformatics

Location: Hall A

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Program#/Poster#: 92.08/BB16

Topic: G.05. Bioinformatics

Title: NeuroGPS-Tree: Automatic reconstruction of a large-scale neuronal population with dense neurites

Authors: *S. ZENG;
Huazhong Univ. of Sci. & Technol., HB, China

Abstract: Tingwei Quan[1,2,3], Hang Zhou[1,2], Jing Li[1,2], Shiwei Li[1,2], **Qingming Luo[1,2], Hui Gong[1,2]*, Shaoqun Zeng[1,2,]*** [1]Britton Chance Center for Biomedical Photonics, Huazhong University of Science and Technology-Wuhan National Laboratory for Optoelectronics, Wuhan 430074, China [2]MoE Key Laboratory for Biomedical Photonics, Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan 430074, China [3]School of Mathematics and Statistics, Hubei University of Education, Wuhan 430205, China To whom correspondence should be addressed. E-mail: huigong@mail.hust.edu.cn, sqzeng@mail.hust.edu.cn **Abstract** The mapping of neuronal circuits plays an important role in studies of the brain. Automatic reconstruction of neuronal populations, a key step in drawing neuronal circuits, remains a challenge due to the high density of neurites. Here, we show the dense reconstruction of a neuronal population by partially mimicking the strategy of an experienced technician. We progressively approach the right reconstruction by repetitively using statistical information about neuronal morphology at multiple scales. For neuronal populations not resolvable by the latest or best available methods, our method achieved a reliable reconstruction with recall and precision rates of approximately 80%. We also demonstrate the reconstruction of a population of 990 neurons within 3 hours and show the potential to quickly reconstruct large-scale neuronal populations.

Disclosures: S. Zeng: None.

Poster

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Support: NIH R03 EB016923

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NIH AG010124

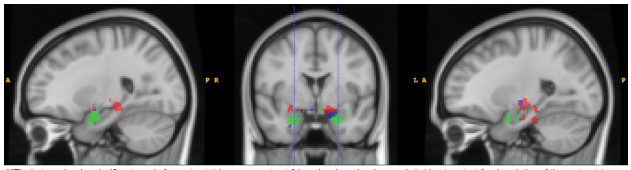
NIH P30 AG010124

NIH AG037376

Title: Dissociable functional activation within MTL during pattern separation measured using high-resolution BOLD fMRI at 7 Tesla

Authors: *S. DAS[1], J. DUDA[2], J. WU[3], M. DAFFNER[4], M. ELLIOTT[5], L. MANCUSO[4], L. WISSE[5], P. YUSHKEVICH[5], D. WOLK[6];
[1]Univ. of Pennsylvania, Bear, DE; [2]Penn Image Computing and Sci. Lab., Univ. of Pennsylvania, Philadelphia, PA; [4]Neurol., [5]Radiology, [3]Univ. of Pennsylvania, PHILADELPHIA, PA; [6]Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Introduction: *In vivo* ultra-high resolution BOLD fMRI of human medial temporal lobe (MTL) at 7T was used to localize functional activation within small MTL subregions. A task operationalized episodic memory constructs of pattern completion/separation and recollection/familiarity, some of which have been shown to be dissociably subserved by different MTL subregions in animal studies, but not yet in humans. **Methods:** Eleven young healthy control subjects were imaged on the Siemens 7T scanner. Imaging included 0.8x0.8x0.8 mm^3 T1-MRI, 0.4x0.4x1.0 mm^3 T2-MRI, 1x1x2 mm^3 GRE-EPI, and a B0 map sequence for distortion correction. In an event-related fMRI paradigm, subjects viewed image pairs of common objects during study phase, and were later shown intact pairs, rearranged pairs, or novel pairs during test phase. They had to endorse only an intact pair as "old", "new" otherwise. Different combinations of trial type and response operationalized different memory constructs, and were modeled in the fMRI data. **Results:** Groupwise analysis examined the following activation contrasts in MTL: 1) Comparison of correct "new" endorsements of rearranged pairs vs. "old" endorsements of intact pairs, 2) Comparison of correct "new" endorsements of rearranged pairs vs. "new" endorsements of novel pairs. Based on models of pattern separation/completion and the underlying MTL substrates, these two contrasts were expected to produce dissociable activations within the hippocampus/MTL. The first condition resulted in primarily anterior clusters (total volume 9984 mm^3), and the second in primarily posterior clusters (total volume 4800 mm^3), while the volume of overlap between them was 824 mm^3 (see figure). **Conclusions:** Our study demonstrates the ability to dissociate functional activation between the anterior and posterior aspects of the hippocampus/MTL depending on the specific memory constructs under comparison. Finer scale assessment using hippocampal and MTL cortex subregions defined in high-resolution MRI may help further elucidate the substrates of episodic memory constructs.



MTL clusters showing significant voxels for contrast 1 in green, contrast 2 in red and overlapping voxels in blue (see text for description of the contrasts).

Disclosures: **S. das:** None. **J. Duda:** None. **J. Wu:** None. **M. Daffner:** None. **M. Elliott:** None. **L. Mancuso:** None. **L. Wisse:** None. **P. Yushkevich:** None. **D. Wolk:** None.

Poster

092. Bioinformatics

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 92.10/BB18

Topic: G.05. Bioinformatics

Support: BMBF Grant 01GQ1302

Title: Consistent data organization made easy: Versatile format for data and metadata

Authors: **A. STOEWER**[1], C. J. KELLNER[1], A. SOBOLEV[1], M. SONNTAG[1], J. BENDA[2], *T. WACHTLER[1], J. GREWE[2];
[1]Ludwig-Maximilians-Univ Munich, 82152 Planegg-Martinsried, Germany; [2]Eberhard Karls Univ., 72076 Tuebingen, Germany

Abstract: Managing neuroscience data requires the integration of information from multiple sources. Different types of recordings may be combined with elaborated stimulation, requiring background information or metadata to interpret them correctly. Storing such information consistently is an essential part of experimental research and depends crucially on available file formats. Many existing formats are vendor or domain specific, or provide only limited support for storing metadata along with the data. Here we present the NIX format [1], an open file format that is versatile enough to represent various kinds of data in conjunction with metadata to facilitate data organization and data retrieval in the lab, as well as data sharing. The format is compliant with the INCF requirements for storing electrophysiology data [2]. It enables storing recorded or derived data as well as all the meta-information about the experimental context, accounting for the relationships between data items. Data arrays are defined with units and dimension descriptors, so that the stored data can be readily interpreted as recorded quantities. The format further enables specifying the relationships between the data arrays and to describe points or regions of interest, such as areas in an image or events in a recorded signal. The NIX software libraries support direct access to these targeted parts of the data and the linked

metadata. This enables selecting specific subsets of the data, such as signals recorded from a certain electrode channel, or spike trains from a certain single unit in trials with a given stimulus. NIX stores data and metadata using the HDF5 format [3]. While it is possible to read and write these files using the standard HDF5 libraries, efficient use of the features of the NIX format is facilitated by specific libraries provided for different languages, including C++, Python [4], Matlab [5], and Java. Packages and installers for different platforms are provided, as well as detailed documentation, examples, and tutorials [6]. The NIX file format supports comprehensive annotation and efficient organization of neuroscience data, and the variety of libraries makes it easy to integrate access to data and metadata in the lab data collection and analysis workflow. [1] https://github.com/G-Node/nix [2] https://incf.org/activities/our-programs/datasharing [3] http://hdfgroup.org/HDF5/ [4] https://github.com/G-Node/nixpy [5] https://github.com/G-Node/nix-mx [6] http://g-node.github.io/nixpy

Disclosures: **A. Stoewer:** None. **C.J. Kellner:** None. **A. Sobolev:** None. **M. Sonntag:** None. **J. Benda:** None. **T. Wachtler:** None. **J. Grewe:** None.

Poster

092. Bioinformatics

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Program#/Poster#: 92.11/BB19

Topic: G.06. Computation, Modeling, and Simulation

Support: NSERC grant 223239

China NSF 61170048

NIH R01MH086638

NIH T15LM007056

Title: Parallel Reaction-Diffusion simulation in NEURON

Authors: ***C. TROPPER**[1], L. ZHONGWEI[2,3], R. A. MCDOUGAL[4], M. HINES[4], W. LYTTON[5];
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Abstract: We previously developed a stochastic parallel discrete event simulator (Neuron Time Warp), NTW was interfaced with 1D and 3D deterministic reaction diffusion simulators within NEURON. The objective of using stochastic simulation is to portray the variable effects of a small number of molecules in, for example, a dendritic spine. We have developed a threaded simulator, NTW-MT. The reason for developing the threaded version is to take advantage of multi-core architectures employed in parallel machines so that we can increase the speed of a simulation and to be able to simulate larger models. One thread takes care of communications while the others take care of computation. Communication between threads makes use of shared memory if the threads are in the same process and by MPI if they are in different processes. A multi-level queueing stucture is employed for the priority queue. Both NTW and NTW-MT make use of a stochastic simulation algorithm, the next sub-volume method (NSM), which is an outgrowth of the Gillespie algorithm. We simulated a discrete event model of a Ca wave on an un-branched apical dendrite of a hippocampal pyramidal neuron on both NTW and NTW-MT. The model we used was derived from a deterministic model (1). NTW-MT scaled well with the size of the model and its execution time scaled well with the number of cores. It performed much better then the process-based NTW as well as a multi-threaded version of NTW which did not employ its multi-level priority queue. While our results indicated that execution time scaled well with the number of processors, the number of rollbacks also increased and caused the decrease in execution time to flatten. Both dynamic window management and dynamic load balancing are necessary in order to contain the number of rollbacks. The window size controls the optimism of Time Warp, preventing an excessive number of rollbacks. We have previously developed AI based algorithms for dynamic load balancing and window management-we are going to implement them in NTW-MT (simulated annealing, multi-state Q-learning and genetic algorithms) shortly. (1) S. A. Neymotin, R. A. McDougal, M. A. Sherif, C. P. Fall, M. L. Hines, and W. W. Lytton. Neuronal calcium wave propagation varies with changes in endoplasmic reticulum parameters: A computer model. Neural Computation, 27(4):898–924, Mar. 2015.

Disclosures: C. Tropper: None. **L. Zhongwei:** None. **R.A. McDougal:** None. **M. Hines:** None. **W. Lytton:** None.

Poster

092. Bioinformatics

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 92.12/BB20

Topic: G.05. Bioinformatics

Support: Ivana received IBRO-SFN travel award

Rao KS received SNI grant from SENACYT, Panama

Melo Brain Grant, Panama

Title: Molecular Networking of oxytocin in Brain- relevance to Alzheimer's pathology

Authors: *I. TEJADA, J. KOSAGISHARAF;
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Abstract: Alzheimer's disease (AD) is a complex neurological disorder with several unequivocally identified genetic risk factors. Among the several environmental factors proposed for AD, dietary molecules act as protective and also risk factors have been most compelling. In particular, diets rich in saturated fatty acids and deficient in antioxidants and vitamins appear to promote the onset of the disease, while diets rich in unsaturated fatty acids, vitamins, antioxidants, and wine likely suppress its onset?. Evidence suggests that diets rich in polyphenols and some spices suppress the onset of AD by scavenging free radicals and preventing oxidative damage. Further, Metal ions are known to catalyze the production of free radicals and induce neurodegeneration. Several studies have also identified metals such as Pb, Fe, Al, Cu and Zn play a role in AD pathogenesis. Metals, Oxidative stress, decrease in antioxidants etc, could possibly lead to AD pathology. We are investigating biological molecules present in the body which can manipulate the above biochemical complex situation. We developed theoretical model to understand the role of responsive molecules namely oxytocin in modulating Alzheimer's disease pathology. We developed networking models to understand how oxytocin interacts with different pathological events and develop protection strategy against neurodegenerative pathways. We used data mining, cluster model and predictive interactive pathway models. The focus is on how oxytocin modulates hypothalamus stimulation, APP processing, abeta aggregation modulations, overcoming oxidative stress and providing a clue on emotional failures etc. Using networking model data, we propose the preventive/therapeutic potential of oxytocin for Alzheimer's diseases (Ivana is awarded IBRO-SFN travel award 2015 and Rao KS is supported by SNI grant of SENACYT, Panama and this work is supported by Melo Brain Grant, Panama).

Disclosures: I. Tejada: None. **J. Kosagisharaf:** None.

Poster

092. Bioinformatics

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 92.13/BB21

Topic: G.05. Bioinformatics

Support: EP/F500385/1

BB/F529254/1

Title: Improving accuracy of site-specific selection pressure analysis of deeply conserved post-synaptic proteins

Authors: *M. PAJAK[1], C. R. BRAMHAM[2], T. I. SIMPSON[1,3];
[1]Sch. of Informatics, Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Dept. of Biomedicine, Univ. of Bergen, Bergen, Norway; [3]BIOSS, Edinburgh, United Kingdom

Abstract: Sequence conservation analysis of the post-synaptic proteome (PSP) revealed that some of the key synapse protein classes emerged in primitive organisms prior to the development of the nervous system. A common procedure in molecular phylogenetics is to use all available ortholog sequences of a target gene to construct a multiple sequence alignment (MSA). The number of annotated genomes grows constantly and this trend is likely to accelerate in the years to come. However, distribution of available annotated genomes on the tree of life is biased towards complex vertebrate organisms, especially mammals, which in turn biases the availability of orthologs for MSA. Here we have quantified the influence of this bias on the downstream phylogenetic analysis, specifically on site specific selection pressure estimates. In parallel we have tested the effect of eliminating poorly aligned columns of MSA which had been previously shown to improve accuracy of the analysis. In our study we discuss the overarching question of whether reducing the size of MSA (in both dimensions) can improve its informativeness for detecting sites under positive/negative selection pressure. Finally, we show the application of our benchmark workflow to a set of deeply conserved elements of PSP in a domain-centric selection pressure analysis setting.

Disclosures: **M. Pajak:** None. **C.R. Bramham:** None. **T.I. Simpson:** None.

Poster

092. Bioinformatics

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 92.14/BB22

Topic: G.05. Bioinformatics

Support: Falk Foundation

Title: A novel approach to study network interactions in cortical cultures

Authors: A. WILDEMAN[1,2], J. E. JURELLER[3], J. C. WANG[1], J. D. MARKS[1], *W. VAN DRONGELEN[4,2];
[1]Dept. of Pediatrics, [2]Committee on Computat. Neurosci., [3]Inst. of Biophysical Dynamics NanoBiology Facility, Univ. of Chicago, Chicago, IL; [4]Dept Pediatrics, Univ. Chicago, Chicago, IL

Abstract: The difficulty in relating macroscopic, emergent phenomena to microscopic properties is a central problem in neuroscience, both in network physiology (e.g. memory, learning) and pathology (epilepsy, schizophrenia). Network function is determined by properties that emerge from the complex, nonlinear interactions between the nodes in the network and our aim is to develop a novel approach to study the effects of these interactions with high temporal precision, spatial resolution, and spatial range. Our methodology manipulates of neuronal connectivity to explore the effects of low-level network properties on emergent macroscopic behavior. We have designed a system in which cultures of dissociated neurons grown on a multi-electrode array (MEA) can be manipulated by the introduction of real-time feedback loops mimicking additional synaptic connectivity. This is achieved by illuminating a small area of the culture and thereby releasing caged neurotransmitter, in reaction to recorded activity elsewhere in the culture. To create the flexibility required to freely add connectivity anywhere within a culture, we incorporate a digital micromirror device (DMD), an array of many small, individually controlled mirrors. The DMD allows us to dynamically select sections of the culture to be illuminated. The 1024x768 DMD grants a 2 μm resolution when covering a square 1.6 mm MEA. We employ a custom high-power LED light source and have developed an optical setup to generate and guide light to the DMD and subsequently to the MEA. As natural synaptic connectivity occurs on a millisecond timescale, we implement the feedback loops, including spike detection on the MEA electrodes, artificial connectivity and the control of the DMD, on a digital signal processor (DSP), which is a dedicated processor embedded in the MEA data acquisition system and avoids the additional delay and jitter that would be introduced by having a PC control the feedback. Preliminary experiments have illustrated that we can alter the behavior of dissociated cultures of rat hippocampal neurons by feedback alone. Through the combination of MEA, DMD and DSP technologies, we are thus able to dynamically specify network interactions with micrometer and microsecond precision and create the possibility of manipulating connectivity on this scale in order to investigate its effects on emergent network properties.

Disclosures: A. Wildeman: None. **J.E. Jureller:** None. **J.C. Wang:** None. **J.D. Marks:** None. **W. van Drongelen:** None.

Poster

092. Bioinformatics

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Topic: G.05. Bioinformatics

Support: NIH Grant NS050641

NIH Grant NS082104

NIH Grant ES22310

Foglia Family Foundation

Les Turner ALS Foundation

Les Turner ALS Foundation/Herbert C. Wenske Foundation Professor

Ride for Life

Title: Whole exome sequencing identifies new genes associated with sporadic trios in ALS

Authors: K. B. AHMETI[1], *F. FECTO[1], K. B. AHMETI[1], Y. YANG[1], N. SIDDIQUE[1], J. YAN[1], M. PERICAK-VANCE[2], H.-X. DENG[1], Y. MA[3], T. SIDDIQUE[1];
[1]Davee Dept. of Neurol. and Clin. Neurosciences, Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [2]Inst. of Human Genomics, Univ. of Miami, Chicago, IL; [3]Ann & Robert H. Lurie Children's Hosp. of Chicago, Chicago, IL

Abstract: Whole Exome Sequencing has become a new means of identifying mutations in coding regions of the human genome. Fifty families of trios with sporadic sporadic ALS (50 trios), recessive ALS (2 families) were prepared using the Agilent SureSelect 70 MB kit and sequenced in Illumina HiSeq2500. The data were analyzed using a High Power Computing built in house. Open source bioinformatics tools such as BWA, SAMtools, GATK, Picard, and VCF as well as in house developed scripts and analytic models allowed us to identify novel variants. Statistical analysis of the coverage showed that over 99% of the exons were covered in most of the samples with average depth of 120x (range per sample was 70x-210x). An average of 550,000 to 750,000 variants per sample were generated. After filtering them further 400-700 novel variants were identified in the coding region. Subsequently, we focused on identifying common variants among all of the samples. The common variants were verified using conventional Sanger sequencing. Furthermore, to verify the statistical importance of these mutations mathematical modeling in logistic regression analysis was used to identify nuclear, mitochondrial and RNA pathways, which may have direct impact on the disease. We next performed Immunohistochemistry on autopsy tissue samples from patients to explore the

involvement of the identified genes/proteins in disease pathogenesis. In-vitro and *in vivo* cell modeling is currently underway to verify the functionality of identified mutations. Whole Exome Sequencing is a powerful tool that has allowed us to identify mutations in both familial and sporadic cases of neurodegenerative diseases.

Disclosures: K.B. Ahmeti: None. **F. Fecto:** None. **K.B. Ahmeti:** None. **Y. Yang:** None. **N. Siddique:** None. **J. Yan:** None. **M. Pericak-Vance:** None. **H. Deng:** None. **Y. Ma:** None. **T. Siddique:** None.

Poster

092. Bioinformatics

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 92.16/BB24

Topic: G.06. Computation, Modeling, and Simulation

Support: NIH Grant T15 LM007056

NIH Grant R01 MH086638

Title: Hybrid 1d/3d reaction-diffusion in the neuron simulator

Authors: *R. A. MCDOUGAL[1], A. S. BULANOVA[2], M. L. HINES[1], W. W. LYTTON[2,3]; [1]Neurobio., Yale Univ., New Haven, CT; [2]Physiol. and Pharmacol., SUNY Downstate, New York, NY; [3]Kings County Hosp., New York, NY

Abstract: Neuroscience simulators provide a framework for combining experimental observations to make predictions about neurons and networks of neurons that are impossible or impractical to measure directly with current technology. Each simulator necessarily has a domain of applicability. The NEURON simulator, used in over 1500 publications, has traditionally focused on problems dominated by electrophysiology, but we recently introduced a reaction-diffusion module to facilitate the specification of problems of that type. Stochastic 3D simulations are essential for studying highly localized phenomena (e.g. calcium microdomains), but this approach becomes computationally infeasible as the problem size increases, as is necessary for calcium waves which can spread over a large portion of a pyramidal cell's apical dendrite. Using a cartesian mesh and a deterministic solver provides well-understood accuracy and stability, but requires tiny voxels to represent the shape of the dendrites which in turn force small time steps to ensure stability. Tetrahedral meshes can represent the morphology more accurately with less compartments, but these present their own difficulties. To avoid these

problems, we are introducing support for hybrid 1D-3D studies in NEURON. Each section of the morphology may be either simulated in 1D or 3D. Fluxes across a 1D-3D border are based on the concentrations in the end-segment of the 1D domain and on the edge disc of voxels in the 3D domain. We use a model of a propagating calcium wave to illustrate our approach. In the 1D version of our model, the gradient at the edge of the wave is sufficiently gradual that we did not expect significant variation across a dendrite. We verified this expectation with a 3D simulation stimulated by IP3 released at a single point. We then simulated calcium waves on traced pyramidal cells, with most of the dendrites in 1D and the soma and the proximal dendrites in 3D. By simulating the soma in 3D, we observed the wave slow and curve as it entered the soma. The speed of the wave as it enters the soma is a function of the amount of calcium sequestered in the ER, which is itself a function of previous electrical activity. The hybrid 1D/3D simulator provides a convenient and efficient tool for reaction-diffusion simulations that span multiple spatial scales.

Disclosures: R.A. McDougal: None. **A.S. Bulanova:** None. **M.L. Hines:** None. **W.W. Lytton:** None.

Poster

092. Bioinformatics

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 92.17/BB25

Topic: G.05. Bioinformatics

Support: Autism Speaks Denis Weatherstone Predoctoral Fellowship (A.D.)

NIH (MH103392)

Title: Integrating and mining the widespread h3k4me3 epigenomic landscape and cell-type specific regulation in human prefrontal cortex and blood

Authors: *A. DINCER[1,2,3], E. SCHADT[2], B. ZHANG[2], D. GAVIN[4], C. XU[2], J. DUDLEY[2], S. AKBARIAN[3];
[1]Mt. Sinai Med. Sch., New York, NY; [2]Dept. of Genet. and Genomic Sciences, Icahn Sch. of Med. at Mount Sinai, New York, NY Icahn Inst. for Genomics and Multiscale Biology, Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Friedman Brain Institute, Dept. of Psychiatry and Neuroscience,Icahn Sch. of Med. at Mount Sinai, New York, NY, NY; [4]Jesse Brown Veterans Affairs Med. Ctr., Chicago, IL, IL

Abstract: Only few histone modifications have been mapped in human brain. Trimethylated histone H3-lysine 4 is primarily distributed in the form of sharp peaks, extending in neuronal chromatin on average only across 500-1500 base pairs mostly in close proximity to annotated transcription start sites. However, it remains unclear whether a subset of H3K4me3 peaks could extend across much broader domains. Here we use next generation sequencing based methods to profile the epigenomic landscape of human, chimpanzee, and macaque prefrontal cortex (PFC) neurons from subjects with healthy controls and provide an innovative approach to identify broadest domain cell-type specific H3K4me3 peaks in neuronal and nonneuronal cells from prefrontal cortex (PFC) - a brain region that contributes to cognitive abilities unique to human - in comparison to nucleated blood cells in an unbiased manner. In PFC neurons, broadest peaks ranged in size from 3.9 to 12kb, with extremely broad peaks (~10kb or broader) related to synaptic function and GABAergic signaling (DLX1, ELFN1, GAD1, LINC00966). Broadest neuronal peaks showed distinct motif signatures, and were centrally positioned in prefrontal gene-regulatory networks. Approximately 120 of the broadest H3K4me3 peaks in human PFC neurons, including many genes related to glutamatergic and dopaminergic signaling, were fully conserved in chimpanzee, macaque and mouse cortical neurons. Exploration of spread and breadth of lysine methylation markings could provide novel insights into epigenetic mechanism involved in neuropsychiatric disease and evolution of neuronal genomes.

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Poster

093. Computation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 93.01/BB26

Topic: G.06. Computation, Modeling, and Simulation

Support: Fellowship, The Rockefeller University

Title: Self-organized information routing and processing in neuronal networks

Authors: *C. KIRST;
The Rockefeller Univ., New York, NY

Abstract: Flexible information routing and context dependent processing fundamentally underlies the function of neuronal networks. How information may be specifically processed and dynamically routed in these systems is however not well understood. Here we show that in

complex networks information can 'surf' on top of collective dynamical reference states. Switching between collective dynamics induces fast and flexible reorganization of information routing between the network's units. We use this principle to show how networks can perform context-dependent processing in a self-organized way. In oscillatory Hopfield-Networks we show how multi-modal pattern recognition and context-dependent processing can be implemented by making use of collective network dynamical states. Our work more generically shows how collective reference dynamics can be assigned an important functional role during information processing and pattern recognition tasks. Our results have applications for the design and learning of information routing in neuronal networks where collective dynamics co-occurs with a communication function and the data analysis of recordings of neuronal network activity.

Disclosures: C. Kirst: None.

Poster

093. Computation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 93.02/BB27

Topic: G.06. Computation, Modeling, and Simulation

Support: nstitute for Collaborative Biotechnologies through grant W911NF-09-0001 from the U.S. Army Research Office and the GE/NFL Head Health Challenge

Title: Life after streamlines: Describing structural connectivity with Reeb graphs

Authors: *M. CIESLAK[1], J. SUN[2], L. VOLZ[3], S. SURI[2], S. T. GRAFTON[3];
[1]Psychological and Brain Sci., UCSB, Santa Barbara, CA; [2]Computer Sci., [3]Psychological and Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Noninvasive study of white matter pathways in the human brain is typically achieved by performing fiber tracking on diffusion-weighted MRI. Fiber tracking produces a set of streamlines, which are arrays of 3D coordinates that in most cases trace the paths of axons through white matter. While visually compelling, streamlines can overestimate connectivity by tracking spurious paths and underestimate connectivity by failing to connect regions when their axons merge and split from a larger bundle. Here we present an algorithm that segments streamlines into coherently-moving “bundles” separated by merging or splitting events. These bundles form a spatial graph-like structure where nodes are merge/split events and edges are bundles. We demonstrate how this graph structure describes motor/premotor connectivity more

accurately than streamlines alone. The reproducibility of bundle segmentations is demonstrated across a cohort of 88 diffusion spectrum imaging streamline datasets.

Disclosures: **M. Cieslak:** None. **J. Sun:** None. **L. Volz:** None. **S. Suri:** None. **S.T. Grafton:** None.

Poster

093. Computation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 93.03/BB28

Topic: G.06. Computation, Modeling, and Simulation

Support: KAKENHI 25120011, JSPS, Japan

Title: Reliability at a macroscopic level in recurrent neural networks: An approach based on canonical correlation analysis

Authors: *H. SUETANI;
Oita Univ., Oita, Japan

Abstract: Since the seminal work by Mainen and Sejnowski, how neural activities are *reliable* against stimuli is an important topic in neuroscience from the viewpoints of transmission of information and neural coding. It has been widely known that in the single cell level, spike timings are reproducible over different trials under the influence of noisy stimuli whereas constant stimuli lead to imprecise spike trains. In this paper, we study how the information of the stimuli from the outside can be maintained in neural activities as a *macroscopic* behavior, i.e., not in the single cell level but in the network level using computational neural network models. Preliminary results are shown in the below. Figure 1(a) shows several time series of three different recurrent neural networks (network connectivity is different each other) with 10^3 neurons subject to the same periodic input with small amplitude. Here, no one can see any reproducibility within the single as well as across different networks. How about the dynamics of a macroscopic level? Figure 1(b) shows time series of the first components of Principle Component Analysis (PCA) for three networks (different colors indicate different networks). No one can see any reproducibility in these again. On the other hand, when we apply *Canonical Correlation Analysis (CCA)*, we can see the same time series with the input periodic signal in the corresponding first components of CCA as shown in Fig. 1(c). PCA and CCA are methods of projections from high-dimensional data to lower-dimensional data. This result means that reproducible activities are hidden in the network level even if the stimulus is tiny and can be

detected by choosing a suitable direction of the projection. We will discuss further results in the presentation.

Disclosures: H. Suetani: None.

Poster

093. Computation

Location: Hall A

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Program#/Poster#: 93.04/BB29

Topic: G.06. Computation, Modeling, and Simulation

Support: NARSAD Young Investigator

Simons Collaboration on the Global Brain

NIH 1U01NS090541-01 (Mechanisms of Neural Circuit Dynamics in Working Memory)

Title: Network models of sequence generation and memory

Authors: *K. RAJAN[1], C. D. HARVEY[3], D. W. TANK[2];
[2]Princeton Neurosci. Inst., [1]Princeton Univ., Princeton, NJ; [3]Harvard Med. Sch., Boston, MA

Abstract: Cellular-resolution imaging of neural activity in PPC during a virtual memory-guided 2-alternative forced choice task [Harvey, Coen and Tank, 2012] has showed that individual neurons had transient activation staggered relative to one another in time, forming a sequence spanning the entire duration of the task. Motivated by these results, our goal here is to develop a computational framework that reconciles the emergence of biologically realistic assemblies or trajectories of activity states, with the ability of the same neural population to translate sensory information into long time-scale behaviors. We build an echo state network to test our hypothesis that during memory-based decision making, sensory cues set up an initial network state that follows the intrinsic dynamics of the brain area to generate activity underlying a behavioral response. We start with a firing rate network which exhibits rich ongoing dynamics correlated over numerous time scales. This network acts as a dynamic reservoir whose modes can be tapped to perform the task through minimal reconfiguration or partial in-network training (PINning), and not complete rewiring. During PINning, only the outgoing weights carrying the synaptic inputs from a subset of neurons in the network are subject to change. There is no external unit that feeds back network output, no learning of readout weights. The learning rule targets only as many units as required for accomplishing the task, the fraction varying with task demands. We show that the PINned network performs a timing task involving a sensory cue, its storage in

working memory during a delay period, and response to its retrieved trace. We change the fraction of trained units until the duration and shape of the sequential activation pattern in the network is comparable with PPC neurons imaged during the task. Further, we show that like the PPC, the network's activity is specific to cue/outcome pairing, temporally confined to a task epoch, and contains similar levels of extra-sequential noise. Notably, since no sequence-specific wiring diagram is embedded a priori units remain spatially intermixed, like the PPC, where there is no topological organization of active neurons. Finally, we study the properties and functional consequences of the synaptic connectivity matrix which is initially random but acquires non-normal features because of PINning. We are currently exploring whether such partially trained networks can be extended to simulate our ability to generalize across different task conditions.

Disclosures: K. Rajan: None. **C.D. Harvey:** None. **D.W. Tank:** None.

Poster

093. Computation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 93.05/BB30

Topic: G.06. Computation, Modeling, and Simulation

Support: NSF-DMS-1313225

Title: Probabilistic synaptic transmission and spike correlations in recurrent cortical networks

Authors: *M. LEONE[1], G. OCKER[2], B. DOIRON[2];
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Abstract: The origin of correlated spiking observed in cortical networks is currently unclear. Theoretical work shows that for large, dense networks, co-fluctuations of strong excitation and inhibition cancel one another, resulting in a network-wide asynchronous state of low mean spiking correlations. Most models of cortical networks tacitly assume that synaptic transmission is infallible, despite a wealth of experimental work to suggest otherwise. In this work, we study the asynchronous state of recurrent cortical networks where the probabilistic nature of synaptic transmission is taken into account, with a focus on the failure of transmitter release. As expected, synaptic failure of inhibition increases the mean firing rate and variability of spiking, even when the trial-averaged output of synapses for a given presynaptic spike train is held constant. However, in dense networks we also see a significant increase in the mean spiking correlations of the network, a result of the fluctuations in inhibitory currents becoming less able to track and cancel fluctuations in excitatory currents. This is counter-intuitive since the failure of synaptic

transmission is independent across synapses, and is at first glance a source of private fluctuations within the network. Our results suggest that the combination of synaptic transmission failure rates and the placement within the recurrent cortical circuit determine the impact of an inhibitory neuron subtype on network correlations. Finally, our results highlight the importance of considering the inherent stochasticity of synapses when studying the statistical activity of dense, recurrent networks.

Disclosures: **M. Leone:** None. **G. Ocker:** None. **B. Doiron:** None.

Poster

093. Computation

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Program#/Poster#: 93.06/BB31

Topic: G.06. Computation, Modeling, and Simulation

Support: NSERC (BA,SP,CC)

Alzheimer's Society of Manitoba Graduate Fellowship (CC)

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Scottish Rite Charitable Foundation of Canada (CC)

The Everett Endowment Fund (BA)

The Edwards Family (BA)

Title: Development of a mathematical model of mitochondrial bioenergetics in cortical neurons

Authors: ***C. CADONIC**[1,6,2], E. THOMSON[1,6], W. SNOW[6], S. ROY CHOWDHURY[6], D. MCALLISTER[6], J. FIEGE[3], P. FERNYHOUGH[4,6], S. PORTET[5,2], B. C. ALBENSI[4,6,2]; [2]Grad. Program in Biomed. Engin., [3]Physics and Astronomy, [4]Pharmacol. and Therapeut., [5]Mathematics, [1]Univ. of Manitoba, Winnipeg, MB, Canada; [6]Div. of Neurodegenerative Disorders, St. Boniface Hosp. Res., Winnipeg, MB, Canada

Abstract: In this project, a mathematical model for mitochondrial function has been developed from oxygen consumption rate (OCR) and oxygen concentration data measured in the Seahorse XF24 Analyzer (Seahorse Biosciences). Measurements in the XF24 Analyzer were conducted on

embryonic-cultured cortical neurons from CD1 mice. Based on the biological mechanism of mitochondrial activity, a deterministic model was developed using biochemical kinetic modelling, and a stochastic validation model was developed using the stochastic simulation algorithm. The deterministic model was calibrated using the optimization genetic algorithm Ferret by fitting real-time OCR data. The model was then coded in MATLAB R2014a (Mathworks) for simulating mitochondrial bioenergetics in silico. To modulate the activity of the mitochondria, specific dysfunctions were introduced by injecting the inhibiting reagents oligomycin, rotenone, and antimycin A; and the uncoupling reagent carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), during OCR measurements. To appropriately incorporate these changes, the model equations were adapted and then re-calibrated to the data for each biological condition. This indicated the flexibility of the model in addressing changes in the biological environment and the appropriate mitochondrial response. The model developed thus maintains the capacity to be expanded upon when calibrated to additional data sets, affording the ability to determine the mechanism of the kinetic changes required to represent biological results.

Disclosures: C. Cadonic: None. **E. Thomson:** None. **W. Snow:** None. **S. Roy Chowdhury:** None. **D. McAllister:** None. **J. Fiege:** None. **P. Fernyhough:** None. **S. Portet:** None. **B.C. Albensi:** None.

Poster

093. Computation

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Support: U.S. Office of Naval Research grant N00014-13-1-0211

U.S. NIBIB grant P41 EB001978

U.S. NIH grant 1U01GM104604

Title: Critical role of topography in determining spatio-temporal network dynamics in a large-scale model of hippocampus

Authors: *P. HENDRICKSON[1], G. J. YU[2], D. SONG[2], T. W. BERGER[2];
[2]Biomed. Engin., [1]USC, Los Angeles, CA

Abstract: This abstract describes a million-plus granule cell compartmental model of the rat hippocampal dentate gyrus, including excitatory, perforant path input from the entorhinal cortex, and feedforward and feedback inhibitory input from dentate interneurons. The model includes experimentally determined morphological and biophysical properties of granule cells, together with glutamatergic AMPA-like EPSP and GABAergic GABAA-like IPSP synaptic excitatory and inhibitory inputs, respectively. Each granule cell was composed of approximately 200 compartments having passive and active conductances distributed throughout the somatic and dendritic regions. Modeling excitatory input from the entorhinal cortex was guided by axonal transport studies documenting the topographical organization of projections from subregions of the medial and lateral entorhinal cortex, plus other important details of the distribution of glutamatergic inputs to the dentate gyrus. Information contained within previously published maps of this major hippocampal afferent were systematically converted to scales that allowed the topographical distribution and relative synaptic densities of perforant path inputs to be quantitatively estimated for inclusion in the current model. Results showed that when medial and lateral entorhinal cortical neurons maintained Poisson random firing, dentate granule cells expressed, throughout the million-cell network, a robust, non-random pattern of spiking best described as spatio- temporal "clustering". To identify the network property or properties responsible for generating such firing "clusters", we progressively eliminated from the model key mechanisms such as feedforward and feedback inhibition, intrinsic membrane properties underlying rhythmic burst firing, topographical organization of entorhinal afferents. Findings conclusively identified topographical organization of inputs as the key element responsible for generating a spatio- temporal distribution of clustered firing. These results uncover a functional organization of perforant path afferents to the dentate gyrus not previously recognized: topography-dependent clusters of granule cell activity as "functional units" or "channels" that organize the processing of entorhinal signals. This modeling study also reveals for the first time how a global signal processing feature of a neural network can evolve from one of its underlying structural characteristics.

Disclosures: **P. Hendrickson:** None. **G.J. Yu:** None. **D. Song:** None. **T.W. Berger:** None.

Poster

093. Computation

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Program#/Poster#: 93.08/BB33

Topic: G.06. Computation, Modeling, and Simulation

Support: Loyola University Research Grant Support

Title: Biomechanics of membrane deformation under time-varying magnetic field - a shell model

Authors: *H. YE[1], A. CURCURU[2];
[1]Dept. of Biol., Chicago, IL; [2]Departmet of Physics, Loyola Univ. Chicago, Chicago, IL

Abstract: Background: Cells deform in a strong electric field (electrodeformation), suggesting an interesting control of cellular biomechanics by the electric field. An alternative method used to generate an electric field is by a time-varying magnetic field. References reporting the magnetic control of cellular mechanics have recently emerged. However, theoretical analysis of the cellular mechanics under a time-varying magnetic field is inadequate. Methods: We have previously developed an analytical theory to investigate the biomechanics of a modeled cell under a time-varying magnetic field (Ye and Curcuru, 2015). For computational reason, the model did not explicitly consider the capacitive properties of the membrane, which represents a complex boundary between the medium and the cytoplasm. In this work, we developed a shell membrane model to include the low-conductive, capacitive cell membrane. We provided detailed analytical solutions for the surface charges, electric fields and radial pressure across the membrane under a time-varying magnetic field. Frequency response of these measures were analyzed, especially for the frequency used in transcranial magnetic stimulation (TMS). Results: The induced surface charges interacted with the electric field to produce a biomechanical impact upon the membrane. The distribution of the induced surface charges depended on the orientation of the coil and field frequency. The direction of the radial force is a function of both the field frequency and the conductivity ratio (cytoplasm/medium). At low frequency, the deformation (pull the cells into a prolate shape or compress into oblate shape) purely dependents on the conductivity ratio. At relative high frequency, cell shape can transit from polate to oblate, for the low conductivity cases. Conclusions: This work provides an analytical framework and insights into factors affecting cellular biomechanics under a time-varying magnetic field. The analysis reveals many unique features of cellular biomechanics under time-varying magnetic field, in comparison with electric stimulation.

Disclosures: H. Ye: None. **A. Curcuru:** None.

Poster

093. Computation

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Topic: G.06. Computation, Modeling, and Simulation

Support: DHHS/NIH 5U54CA132378-07

DHHS/NIH 5R03EB017410-02

Title: Factors influencing current flow through the skin during transcranial electrical stimulation: role of waveform, tissue properties, and macro-pores

Authors: *N. KHADKA[1], B. GULEYUPOGLU[2], D. Q. TRUONG[2], V. PATEL[2], C. THOMAS[2], O. SEIBT[3], A. MOKREJS[2], M. BIKSON[2];
[1]Biomed. Engin., [2]City Univ. of New York, City Col., New York, NY; [3]City Univ. of New York,City college, New York, NY

Abstract: Fundamental questions remain about the mechanisms of transdermal current passage during transcranial electrical stimulation (tES) and approaches to optimize electrodes, waveform, and skin preparation to maximize tolerability. Examples of previously unexplained observations include 1) a higher impedance to DC (transcranial Direct Current Stimulation, tDCS) than to high-frequency stimulation, 2) skin lesions occur rarely at the electrode edges where prior models predict maximum current density, 3) abrasion appears to decrease tolerability in some instances (e.g. tDCS) and enhance it in others (e.g. Electroconvulsive therapy, ECT), 4) repeated stimulation sessions may enhance tolerability or increase irritation, 5) preference for gel or saline properties appears to vary across applications. We developed the first high-resolution FEM model of skin comprising sweat pores, epidermis, dermis, sweat glands, subcutis, hair follicles, and blood vessels. An analytical solution for skin permeability based on cylinders filled with saline to mimic sweat pores was also developed. We conducted experiments testing varieties of electrode assembly and characterized skin and gel response. Under the conditions tested, skin irritation reflects current passage rather than electrochemical burden. During tDCS, the epidermis present a significant captive barrier and current is predominately carried by sweat pores such that moisturizing the epidermis but not abrasion will increase tolerability. Regions of susceptibility are thus near larger sweat pores and not at electrode edges. During tES with higher frequencies, epidermal impedance decreases reducing required stimulation voltage and increasing tolerability. Thus depending upon the waveform applied, specific electrode design and skin preparation techniques can be rationally designed to enhance tolerability.

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Poster

093. Computation

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Program#/Poster#: 93.10/BB35

Topic: G.06. Computation, Modeling, and Simulation

Title: Quantitative analysis on the impacts of dendritic morphology on neuron electrophysiology simulations

Authors: X. LIU, N. GOUWENS, Z. ZHOU, M. HAWRYLYCZ, C. KOCH, A. ARKHIPOV, *H. PENG;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: With the advent of semi-automated neuronal reconstruction techniques, neuroscientists have begun quantitatively analyzing morphometry at a large scale [NeuroMorpho.Org,Human Brain Project, BigNeuron] to investigate the diversity of the dendritic arborization and to provide input for neuronal simulations. Several studies have suggested that dendritic morphology directly influences neuronal firing activities [Mainen & Sejnowski, Nature 96']. However, how specific alterations in morphology affect electrophysiology modeling and simulation outcomes is still not well understood. Here we quantify the effects of dendritic morphology alterations on neuronal electrical behaviors in simulations based on single-cell electrophysiological recordings. As part of the Allen Cell Types Database, we have collected both the 3D neuronal morphologies and the corresponding physiology recordings for a number of individual cells representing a wide variety of neuronal types in the mouse cortex. We first use the recorded responses to subthreshold current injections to estimate three basic passive electrical parameters (intracellular resistivity, membrane resistivity and membrane capacitance) for each neuron via a model-fitting procedure. We then alter the morphologies by changing the diameters, length, and branch topology systematically and re-fit the electrical parameters with the newly-generated morphologies. We quantify the statistical differences in the estimated passive parameters to reveal the sensitivity to particular morphological adjustments. We also use active models of neurons (optimized to match the firing patterns of the recorded neurons) to investigate their sensitivity to these same alterations in morphology. Our study provides quantitative insights into how morphology variations affect physiological response in simulations as well as into the level of accuracy of morphological reconstructions needed for reliable physiology simulations.

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Poster

093. Computation

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Topic: G.06. Computation, Modeling, and Simulation

Support: International Group of Neuroscience (Independent, Multi-institutional Support). For ethical, peaceful, non-commercial and responsible applications of Neuroscience (Grant Philosophy: ¨Science for exploration, not for domination").

Title: Reestablishing Ca^{2+} amplitude and speed during deep sleep in Alzheimer's disease with EEG-triggered TES/TMS: neuromodulation of abnormal columnar states

Authors: *J. F. GOMEZ-MOLINA[1], U. M. RICOY[3], J. VELEZ-M.D.[2], D. SEPULVEDA-FALLA[4];
[1]Intl. Group of Neurosci., Medellin, Colombia; [2]USA member, Intl. Group of Neurosci., New York, NY; [3]Biol., New Mexico Col., Northern New Mexico Col. NM, Española, NM; [4]Inst. of Neuropathology-, Univ. Med. Ctr. Hamburg-Eppendorf and Neurosci. Group of Antioquia, Fac. of Medicine, Univ. of Antioquia, Medellin, Colombia., Hamburg, Germany

Abstract: -INTRODUCTION. (1) Recent evidence shows that familial Alzheimer´s disease (AD)-associated presenilin-1 mutations alters calcium homeostasis in cellular models (http://www.ncbi.nlm.nih.gov/pubmed/22842534) and in fAD patients (Sepulveda-Falla, 2014), leading to neurodegeneration. (2) Previous computer simulations have suggested that in AD there are changes in the waveform of [Ca]i (increase in amplitude and speed) and this also occurs in deep sleep (Gomez 2003a,b). (3) *In vitro* experiments suggest that Ca-Channels have a frequency dependent behavior (Ricoy and Frerking 2014). (4) EEG-triggered TMS/TES (ETT) can amplify (or reduce) the amplitude of Calcium signaling (Gomez-Molina, SfN 2014). Here, we study the biophysical basis of this stimulation. -METHODS. Math-computer models. -THEORETICAL RESULTS. ETT can modify the amplitude and apparent speed of electrodiffusion of Ca2+ in a columnar structure. Fig. 1 -DISCUSSION. (1) If AD patients show increases in Ca2+-amplitude during wakefulness, should we reduce this amplitude during deep sleep or increase it? (2) Unpredictable effects of electrodiffusion of [Ca2+]o and effects on astrocytes should also be analyzed.(3) Experimental work is also required. -CONCLUSIONS. (1) Using ETT we can increase the Ca2+-amplitude and speed in macrocolumns with opposite local states to global EEG. This might improve function. (2) If local and global sleep present similar dynamics then reductions in amplitude can be expected during wakefulness after stimulation during sleep. (3) Weak and friendly stimulation is needed to avoid damage of delicate macromolecular nets around Ca-channels. (4) Presenilin mutations effect on Ca2+ signaling is characterized by increased intracellular levels mostly surrounding the ER, eventually affecting synaptic activity. This effect could result in excitotoxicity. Hence, alternatively, it could be desirable to reduce Ca2+ waveform amplitude by TES/TME stimulation in order to compensate ER Ca2+ leakage in neurons.

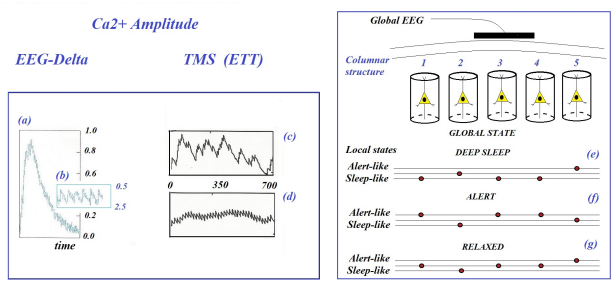

Fig. 1. Computer simulation. Ca 2+-amplitude is higher for bursting linked to EEG-delta (a) or local sleep in a columnar structure, than to tonic neural spiking associated to states of lower EEG-amplitude (b).

Computer simulation of the effects of EEG-triggered TMS (c) vs. TMS alone (d) in Ca2+-amplitude.

EEG-triggered TMS/TES (ETT) can be applied in a global state of deep sleep (e) when many columnar structures are in local sleep (columns 1, 3 and 4) but it can also be applied in global states of alert (f) or relax (g). In all these cases, the goal of stimulation is to change the state of column 2 –a column that is in an abnormal or pathological state. Column 5 is in a normal, physiological state, although this state is not of the same than the global state.

For additional details see references to our previous work:

Sepulveda-Falla D, Barrera-Ocampo A, Hagel C, Korwitz A, Vinueza-Veloz MF, Zhou K, Schonewille M, Zhou H, Velazquez-Perez L, Rodriguez-Labrada R, Villegas A, Ferrer I, Lopera F, Langer T, De Zeeuw CI, Glatzel M. (2014) Familial Alzheimer's disease-associated presenilin-1 alters cerebellar activity and calcium homeostasis. J Clin Invest. Apr;124(4):1552-67. doi: 10.1172/JCI66407.

Ricoy Ulises M, Frerking Matthew E (2014) Distinct roles for Ca(v)2.1-2.3 in activity-dependent synaptic dynamics. J. Neurophysiol 111: 2404-2413 doi: 10.1152/jn.00335.2013

Gomez-Molina Ricoy Escobar Velez (2014) How can the open probability of ionic channels and the wave form of a burst be maximized by designing an EEG-triggered sequence of TES/TMS? Poster684.10/A54 Society For Neuroscience Meeting Abstract on line sfn.org
Gomez-M JF, Lopera-R F, Pineda D and Rios A. (2003) Extracellular potentials and [Ca2+]I during development and neurodegeneration. Proceedings 7th International Conference on Cognitive and Neural Systems, Boston University, Boston (MA). P. 104. Gomez-Molina JF (2003) Análisis de Simulación de EEG e imagenología de calcio en el sueño profundo y la vigilia. Revista Neuropsicologia, Neuropsiquiatria y Neurociencias. 5(1), P. 89

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Poster

093. Computation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 93.12/BB37

Topic: G.06. Computation, Modeling, and Simulation

Title: Exploring the effects of spatial constraints on cell network formation in the dentate gyrus

Authors: *A. MERGENTHAL, T. BERGER;
USC, Los Angeles, CA

Abstract: The cross sectional geometry of the dentate gyrus changes dramatically over the septotemporal extent of the hippocampus. This geometry acts as a constraint on the formation of cellular networks between granule cells and the interneurons located within the hilus region of the dentate gyrus. It is not understood how these variations in network formation alter the cellular activity and computational roles of the cells which form these networks. We explored these effects using a large scale computational model of these cell networks. This model simulated the connections between entorhinal cortex cells, granule cells, basket cells, and HIPP cells. The NEURON simulation environment was used to construct this compartmental model. Cell numbers were one tenth of their measured or estimated populations. A three dimensional reconstruction of the rat dentate gyrus provided spatial measurements to constrain both axonal

and dendritic extents. These constraints provided a novel way to restrict connectivity between cells and provided a more anatomically appropriate simulated cell network. A second and third model were constructed each having the same cell numbers, types, and locations but changing the connectivity constraints such that one had no spatial restraints on connectivity while the other had a simple constraint based upon distance along the curve of the granule cell layer. The three alternate networks were then compared to see what effects the anatomical constraints produced in granule cell activity. Using these comparisons we can better understand how septotemporal variation in cross sectional shape effects the regional information processing roles of the dentate gyrus.

Disclosures: **A. Mergenthal:** None. **T. Berger:** None.

Poster

093. Computation

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH Grant U01 GM104604

Title: Computational study of local field potentials in a heterogeneous 3D model of rat hippocampus

Authors: ***K. LOIZOS**[1], J. CLINE[1], G. YU[2], P. HENDRICKSON[2], G. LAZZI[1], T. BERGER[2]; [1]Univ. of Utah, Salt Lake City, UT; [2]USC, Los Angeles, CA

Abstract: A computational study was conducted to analyze different methods of simulating local field potential (LFP) recordings in a rat hippocampus model. Recorded LFPs summate the spatial temporal activity of the subregion surrounding the electrode. By including the tissue heterogeneity and complex behavior of the cellular network, the effect variation of these parameters has on the recorded potentials can be predicted. Multiple computational models of varying complexity were considered in this study, employing a multi-scale approach. A 3D discretized model of a hippocampus slice with multi-electrode arrays placed on the surface was utilized, constructed from a published atlas. It was discretized based on the tissue resistivity, implementing homogeneous properties for each tissue type in the hippocampus. Admittance method simulations were run, applying current sources to the electrode arrays. Generated voltages were then applied as extracellular voltage sources in a multi-cell compartmental model, which includes thousands of morphologically-unique granule cells, complex ionic membrane

channels, and innervating axons from entorhinal cortex. This process provides estimations of the reactive behavior of the network to electrical stimulation. The resulting membrane current due to any activated cells was then used to estimate LFPs at a given distance away, considering two methods. First, an analytical approach was considered, summing the contributions from all compartments in the network. Each value was computed using Ohm's Law, multiplying the membrane current by an approximate resistance based on the distance from the recording point and a uniform resistivity. Second, an Admittance Method simulation was conducted, following the simulation used to calculate the extracellular potential fields based on input current. For this simulation, the membrane currents were applied as the sources in a time-stepping simulation. The resulting potential field was then observed at the locations of recording electrodes. This method was then further expanded, incorporating the complex morphological data in the dielectric properties used to discretize the admittance model. By varying the complexity of the LFP calculations, we are able to compare results of different computational methods and with experimental data. In doing so, we provide data for deciding the necessary amount of computational complexity necessary for accurate predictions. In addition, the added parameters allow us to distinguish the contribution of individual cellular properties on the estimated LFP recordings.

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Poster

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Topic: G.07. Data Analysis and Statistics

Support: Allen Institute for Brain Science

Howard Hughes Medical Institute

International Neuroinformatics Coordinating Facility

The Kavli Foundation

GE

Title: Neurodata Without Borders (NWB) - a common neurophysiology file format

Authors: K. B. GODFREY[1], J. L. TEETERS[2], C. FRIEDSAM[3], B. WARK[3], R. YOUNG[1], J. BERG[1], *S. R. OLSEN[1], G. DENISOV[4], N. LI[5], S. PERON[5], H. ASARI[6], A. PEYRACHE[7], T. BLANCHE[1], A. GARNER[1], J. SIEGLE[1], N. GOUWENS[1], A. ARKHIPOV[1], S. DURAND[1], S. DE VRIES[1], D. FENG[1], M. CHUN[8], K. D. HARRIS[9], K. SVOBODA[5], G. BUZSAKI[7], M. MEISTER[6], C. KOCH[1], C. DANG[1], F. T. SOMMER[2];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Redwood Ctr. for Theoretical Neuroscience, UC Berkeley, Berkeley, CA; [3]Physion LLC, Cambridge, MA; [5]Janelia Farm Res. Campus, [4]Howard Hughes Med. Inst., Ashburn, VA; [6]Caltech, Pasadena, CA; [7]NYU Neurosci. Inst., New York Univ., New York City, NY; [8]The Kavli Fndn., Oxnard, CA; [9]Univ. Col. London, London, United Kingdom

Abstract: Scientific progress is increasingly enabled by data sharing. For example, astronomy and genomics have adopted data standards to enable research on shared data and the development of common data analysis tools. In contrast, most of neuroscience, including cellular-based neurophysiology, still takes place within boundaries of small laboratories and the communication between laboratories is largely by independent research papers. Little data is shared, and if it is so, the lack of a standard format causes labor overhead for customizing tools and hampers the comparison of data across laboratories. In the last 15 years various individual labs and initiatives have attempted to create a common data format for neurophysiology but none have found adoption by the larger community. To address this difficult problem, the Neurodata Without Borders--Neurophysiology initiative started in August 2015 with the goal to develop a common format between four large neurophysiology laboratories: the Buzsaki group at NYU, the Svoboda group at Janelia Farm, the Meister group at Caltech, and the Allen Institute for Brain Science. The result of the project is a common file format that represents a wide variety of neurophysiology data, including single-cell patch clamping, extracellular electrophysiology and optophysiology. The Neurodata Without Borders (NWB) format is implemented in HDF5 and is designed to store data in a self-documenting way. There is HDF5 support in Python, MATLAB and various programming languages including C++, making it a cross-platform solution. The storage schema is described by a specification language and it is extensible to support future scientific needs as well as lab-specific requirements. The format stores metadata about the experiment session and several varieties of time-series data, including measurements from electrodes, events, stimulus data and image stacks. It also stores the results of intermediate processing, such as spike waveforms, clustering data and unit times (electrophysiology), image segmentation and regions of interest (optophysiology) and positions (tracking). The initial NWB draft version has been released and is currently used to share data sets from the NWB collaborators at crcns.org. In addition, the Allen Institute for Brain Science has adopted the NWB format for all physiology experiments, including the electrophysiology characterization of single cells as a part of the publicly released Allen Cell Types Database. The NWB format will also be useful to other laboratories. Technical information about the format can be found at https://github.com/NeurodataWithoutBorders.

Disclosures: **K.B. Godfrey:** None. **J.L. Teeters:** None. **C. Friedsam:** None. **B. Wark:** None. **R. Young:** None. **J. Berg:** None. **S.R. Olsen:** None. **G. Denisov:** None. **N. Li:** None. **S. Peron:** None. **H. Asari:** None. **A. Peyrache:** None. **T. Blanche:** None. **A. Garner:** None. **J. Siegle:** None. **N. Gouwens:** None. **A. Arkhipov:** None. **S. Durand:** None. **S. de Vries:** None. **D. Feng:** None. **M. Chun:** None. **K.D. Harris:** None. **K. Svoboda:** None. **G. Buzsaki:** None. **M. Meister:** None. **C. Koch:** None. **C. Dang:** None. **F.T. Sommer:** None.

Poster

093. Computation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 93.15/BB40

Topic: G.06. Computation, Modeling, and Simulation

Title: Beer-lambert optical law applied to early steps of phototransduction

Authors: ***E. M. SALIDO**, L. SERVALLI, J. C. GOMEZ, C. VERRASTRO;
Natl. Technological Univ. (UTN), Ciudad Autónoma De Buenos Aires., Argentina

Abstract: Phototransduction classical models do not consider the reduction of available rhodopsin when it is activated by light and, consequently, do not account for the absorptance variation of the outer segment in different brightness conditions. The optical Beer-Lambert law describes the relationship between the light absorbed by a medium and its absorptance. This paper implements this concept and the bleaching phenomenon in equations that admit photons per second as input and resolve in active rhodopsins per second. In addition, this work compares the responses, both in active rhodopsins and in photocurrent, between a classical model of phototransduction developed by Forti et al. (1989) and a model proposed in this study. In Forti's equations, the amount of activated rhodopsin vs. light stimulus shows a linear relation while the new model saturates exponentially. As for photocurrent, the new model proved to behave equivalently to the experimental and theoretical data published by Forti in dark-adapted rods, while under light adapted conditions the new model fits significantly better to the experimental results obtained by Forti. This new model describes the early events involved in phototransduction, extends the light dynamical gain and it can be easily implemented in different types and species of photoreceptors.

Disclosures: **E.M. Salido:** None. **L. Servalli:** None. **J.C. Gomez:** None. **C. Verrastro:** None.

Poster

093. Computation

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Topic: G.06. Computation, Modeling, and Simulation

Support: This research was supported in part by grants from the National Institute of Mental Health MH087755 to SN

Title: Single neuron model with multiple biological characteristics

Authors: *F. FENG, A. ALTURKI, A. NAIR, P. SAMARTH, S. NAIR;
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Abstract: Biophysical conductance-based models of neurons incorporate physiological information including morphology, current channels, and synapses. Such single cell models vary in morphological complexity from one to over 1000 compartments. Computational overheads necessitate the use of reduced order single cells in large model neuronal networks. However, in such cases, it is important that the reduced order models selected retain the key biophysical properties of biological cells. We hypothesize that in a single neuron, distinct groups of channels might be responsible for maintaining biological characteristics such as resting potential, sub-threshold oscillations, and spiking behaviors. Such a segregation also reduces the interference between currents and simplifies model development. This hypothesis is tested using a 1-compartmental biophysical model that reproduces the biological characteristics of two different biological neuron types reported in the literature. One of them is a CA3b hippocampal neuron (Hemond et al., 2008) and the other is a pyramidal neuron in the rodent lateral amygdala (LA) (Faber et al. 2001). Furthermore, we compare the characteristics of the LA single compartment model with 3-, 5- and 69- compartment versions previously developed by our team. The proposed single compartment methodology also provides analytical insights into the contributions of channels to the neuronal properties, e.g., frequency of the low- and high-threshold oscillations. We also propose a pre-filter module that simulates dendritic propagation and computation. This pre-filter is attached to the single compartment model to enable interfacing with other neurons in a network. The next step is to validate the proposed single cell model and pre-filter connection for synapses at the network level by replicating the network findings in Kim et al. (2013) that reproduced experimentally observed emergence of two different tone responsive cell populations after a Pavlovian auditory fear conditioning paradigm. That network model includes tone and shock as inputs, and also the effects of neuromodulators. For this validation, the single compartment cell model, together with the pre-filter synaptic

connection will be embedded into a larger 25,000 cell version of the 1000-cell model in Kim et al. (2013).

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Poster

093. Computation

Location: Hall A

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Topic: G.06. Computation, Modeling, and Simulation

Support: PRESTO, Japan Science and Technology Agency (JST)

JSPS KAKENHI Grant Number 15K00391

the Kayamori Foundation of Informational Science Advancement

Title: Analysis of the dependence of spike generation on the past neuronal activity

Authors: *T. YAMANOBE;
Hokkaido University, Sch. of Med., Sapporo, Hokkaido, Japan

Abstract: Information is transmitted by spikes in nervous systems. However, it is not known which statistic of spikes is the information carrier. If information is encoded in the precise pattern of spikes, generation of each spike should not be dependent on the previous activity of the neuron. In artificial neural networks, the inputs to a single neuron model are transformed by an output function and the information carrier in artificial neural networks depends on the selection of the output function. Thus, it is necessary to examine how the input is transformed into the output at the single neuron level. In this study, we examine the dependence of the global dynamics of an impulse-driven stochastic neuronal model with spontaneous firing on the model and the input parameters. Phase transition curve has been used to describe the phase shift due to a single isolated impulse in the deterministic neuronal models with a limit cycle. In the previous study, we derived a stochastic version of the phase transition curve which is a Markov operator and describes the dynamics of the entire phase plane. The Markov operator governs the state density evolution of the stochastic neuronal model. To examine the effect of the relaxation time to the limit cycle on the global dynamics, we derive a Markov operator that describes the stochastic dynamics restricted to the limit cycle. These Markov operators can reproduce the response of the stochastic neuronal model to time-varying impulses. Using these Markov

operators, we investigate the difference between the steady state and the transient responses. For this purpose, we calculate the number of spikes between two consecutive impulses to relate the dynamics of the neuronal model to the measurable quantity. Each Markov operator can be decomposed into stationary and transient components by the mathematical properties. This allows us to evaluate the difference in the number of spikes per unit time between the steady-state and transient responses of the neuronal model. Our analysis shows how the spike generation depends on the past neuronal activity by changing the relaxation time, the noise strength, and the impulsive input parameters. Furthermore, we propose a scheme to check the dependence on the past neuronal activity by experimentally.

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Poster

093. Computation

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Topic: G.06. Computation, Modeling, and Simulation

Support: École des Neurosciences de Paris

DIM Cerveau et Pensée, France

ANR-14-CE13-0003

Title: Origin of the kink of somatic action potentials

Authors: *M. TELENCZUK[1], B. FONTAINE[2], R. BRETTE[1];
[1]Inst. De La Vision, Paris, France; [2]Lab. of Auditory Neurophysiol., Univ. of Leuven, Leuven, Belgium

Abstract: Hodgkin and Huxley (HH) described a model of action potential generation in their pioneering work in 1952, mainly based on experiments in the space clamped (isopotential) squid giant axon. Despite the general success of the HH formalism to model ionic channels across cell types and species, the isopotential HH model fails to account for some aspects of action potentials observed in cortical neurons. Specifically, somatic action potentials rise much faster in real neurons than predicted by the isopotential HH model, which appears as a « kink » at spike onset. Three mutually exclusive hypotheses have been proposed to explain this phenomenon : cooperativity of sodium channels [Naundorf et al., 2006], active backpropagation [Yu et al., 2008] and compartmentalization of spike initiation [Brette, 2013]. We tackled this problem by

computational modelling and theoretical analysis of spatially extended neuron models. We varied systematically the morphology of the neuron and distribution of the ionic channels along the cell, and tested how they contribute to the appearance of the kink. We asked three important questions: 1) How do sodium channels activate as a function of somatic voltage? 2) Is a big soma necessary for the phenomenon? 3) Is active backpropagation necessary? Our analysis reveals that sodium channels open abruptly in the axon initial segment, causing discontinuity in the somatic I-V curve recorded in voltage clamp. We also show that a big soma is necessary for the kink to be present while active backpropagation is not required. Finally, we created a simplified 2-compartmental model that displays the phenomenon, without active backpropagation. We conclude that the fast rise of spikes in experimental recordings is explained by compartmentalization [Brette, 2013] rather than by active backpropagation [Yu et al., 2008]. References [Brette, 2013] Brette, R. (2013). Sharpness of spike initiation in neurons explained by compartmentalization. PLoS Comp Biol [Naundorf et al., 2006] Naundorf, B., Wolf, F., and Volgushev, M. (2006). Unique features of action potential initiation in cortical neurons. Nature, 440(7087):1060-3. [Yu et al., 2008] Yu, Y., Shu, Y., and McCormick, D. a. (2008). Cortical action potential backpropagation explains spike threshold variability and rapid-onset kinetics. J Neurosci, 28(29):7260-72.

Disclosures: **M. Telenczuk:** None. **B. Fontaine:** None. **R. Brette:** None.

Poster

093. Computation

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Topic: G.06. Computation, Modeling, and Simulation

Support: ONR Grant N00014-13-1-0211

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NIH Grant 1U01GM104604

Title: Emergent anatomical and functional organization of place bias cells in the dentate in a large-scale biologically realistic model of the rat hippocampus

Authors: *G. J. YU[1], P. HENDRICKSON[2], D. SONG[2], T. W. BERGER[2];
[2]Biomed. Engin., [1]USC, Los Angeles, CA

Abstract: Grid cell input was incorporated into a large-scale, biologically realistic model of the rat hippocampus to investigate how behaviorally relevant input would be transformed due to the non-linearities and complexities present in the model. By varying levels of GABAergic feedback inhibition, the formation and shape of place bias cells in the dentate gyrus can be modulated. The anatomical organization of grid map properties in the entorhinal cortex and the topographic organization of projections between the entorhinal cortex and dentate gyrus resulted in a functional and anatomical organization of the properties of place bias cells in the dentate gyrus. The dorsal dentate granule cells exhibited place bias over smaller areas while ventral granule cells exhibited larger place bias. At 1/10th of the full scale of cell numbers, the hippocampal model consisted of entorhinal cortex layer II cells, granule cells, and basket cells with over 100,000 neurons and was modeled using NEURON. Granule cell models had individually generated morphologies and incorporated non-uniformly distributed active and passive membrane properties. The basket cells were approximated using a single-compartment model. The neurons were distributed in a space approximating the hippocampal dimensions based on published measurements. The topography of the projection between entorhinal cortex and dentate was constrained using anatomical data, and the connectivity between the granule cells and basket cells were similarly constrained. Excitatory synaptic coupling was mediated through AMPA-like synapses while inhibitory synapses were GABAergic. The entorhinal cortex cells were represented as heterogeneous Poisson spike generators whose time-varying mean firing rates were dependent on the individual grid map of a cell and the location of a virtual rat as it randomly moved in a square environment. The properties of the grid maps were organized along the dorsoventral axis of the entorhinal cortex with grid scales increasing in the ventral direction. The hippocampus serves an important role in the formation of episodic memories. However, it is yet to be fully understood how the hippocampus provides this function. In our large-scale hippocampal model, we were able to confirm several hypotheses about place field generation using biologically-realistic, spiking neurons. By incorporating behaviorally realistic inputs to the large-scale model, we hope to uncover, unknown phenomenon that can assist in confirming current hypotheses and drive the discovery of new hypotheses that can be tested experimentally to finally unravel the hippocampal mystery.

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Poster

093. Computation

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Topic: G.06. Computation, Modeling, and Simulation

Support: P41 EB001978-24

U01 GM104604

Title: Nonlinear modeling of calcium dynamics in glutamatergic postsynaptic spine for large scale simulations

Authors: *E. Y. HU[1], J.-M. BOUTEILLER[1], D. SONG[1], M. BAUDRY[2], T. W. BERGER[1]; [1]Biomed. Engin., USC, Los Angeles, CA; [2]Biomed. Sci., Western Univ., Pomona, CA

Abstract: Calcium in the postsynaptic spine plays a critical role in the activation and regulation of pathways that lead to a wide range of phenomena, from genome transcription to long term potentiation and even excitotoxicity. Postsynaptic calcium concentration is the result of influx from postsynaptic receptors, channels, and regulators within the synapse. Such intricate cellular mechanisms are still not fully understood and constitute an active area of research. The complexity and nonlinear nature of the dynamics make it difficult to elucidate how postsynaptic calcium dynamics can influence large neuron networks as well. Here we have developed an input-output (IO) model that captures the dynamics of a complex, detailed parametric model for postsynaptic calcium. Our goal is to better understand the mechanisms that influence postsynaptic calcium concentration, as well as evaluate the effect of calcium dynamics on a larger scale simulation. The calcium dynamics captured by the IO model consists of influences from both metabotropic and ionotropic glutamate receptors, calcium pumps, and contributions from the endoplasmic reticulum (ER). We show that our model is capable of accurately replicating the dynamics seen in the detailed parametric model with (i) little error and (ii) a substantial gain in computational efficiency, allowing the model to be used in large scale simulations comprising a large number of synapses and neurons. Use of this model may provide further insight into calcium dynamics both on a subcellular level and in a large network of neurons, and provide a better understanding of complex physiological processes such as long term potentiation and neuroplasticity.

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Poster

094. Computation: Other

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 94.01/BB46

Topic: G.06. Computation, Modeling, and Simulation

Support: NIH R01 EB018297

Title: The role of cellular membrane properties in generating synchronous activity in inhibitory networks

Authors: *S. RICH[1], V. BOOTH[2], M. ZOCHOWSKI[3];
[2]Mathematics, [3]Biophysics, [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Inhibition plays a vital role in generating synchrony in neural circuits. The established ING mechanism for generating synchrony in inhibitory networks has been thought of as independent of neural membrane excitability properties. We show this hypothesis does not fully encapsulate the formation of synchrony in inhibitory networks. We analyze synchronization for three different cellular excitability types, and for networks with low and high heterogeneity in the intrinsic cell firing frequency. Large scale, sparsely randomly connected networks were studied with each model. These models are typified by their IF (current-frequency) curve and their PRC (phase response curve), which quantifies the change in phase of the subsequent firing of a neuron in response to current pulses at various phases within its normal periodic firing. Type I neurons have a steep IF curve that can fire at arbitrarily low frequencies and a PRC that always shows phase advance in response to an excitatory pulse. Type II neurons possess a shallow IF curve and a minimum firing frequency, as well as a PRC showing a small phase delay in response to an excitatory pulse soon after firing. We also study Type II neurons with an M-type potassium current which acts as an adaptation current, promoting rebound firing following inhibition and causing the neuron to act more like a Type I neuron in the presence of inhibition. Our simulations in these networks show that Type I cells synchronize via the ING mechanism: full synchrony is observed for sufficiently long lasting synapses in networks with low cell heterogeneity, while high cell heterogeneity promotes partial ING-driven synchrony with full ING synchrony obtained for sufficiently high intrinsic mean cell firing frequencies. However, the picture is more complicated for Type II cells. Type II cells without an adaptation current exhibit spontaneous cluster synchrony in the case of low heterogeneity that can be driven to full ING synchrony, indicating a bistability. With high heterogeneity ING synchrony is not observed, as instead cluster synchrony occurs. Finally, in the Type II model with an adaptation current, networks can display either cluster synchrony or full ING synchrony dependent upon the intrinsic mean cell firing frequencies and the length of synaptic decay with a rapid transition between these regimes, independent of the degree of heterogeneity. These results show that the formation of synchrony in inhibitory networks can be complex and subject to neuromodulation by adaptation currents, and is not fully explained by synaptic properties as the ING theory suggests.

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Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: ARC Centre Grant CE140100007

Title: Dynamic patterns in a two-dimensional neural field with refractoriness

Authors: *Y. QI, P. GONG;
Sch. of Physics, Univ. of Sydney, Sydney, Australia

Abstract: Formation of dynamic patterns such as localized propagating waves is a fascinating self-organizing phenomenon that happens in a wide range of spatially extended systems including neural systems, in which they might play important functional roles. Here we derive a type of two-dimensional neural field model with refractoriness to study the formation mechanism of localized waves. The model is able to generate a variety of localized patterns, including stationary bumps, localized waves rotating along a circular path, and localized waves with long-range propagation. We construct explicit bump solutions for the two-dimensional neural field, and conduct a linear stability analysis on how a stationary bump transitions to a propagating wave under different spatial eigenmode perturbations. The neural field model is then partially solved in a co-moving frame to obtain localized wave solutions, whose spatial profiles are in good agreement with those obtained from simulations. We demonstrate that when there are multiple such propagating waves, they exhibit rich propagation dynamics, including propagation along periodically oscillating and irregular trajectories; these propagation dynamics are quantitatively characterized. In addition, we show that these waves can have repulsive or merging collisions, depending on their collision angles and the refractoriness parameter. Due to its analytical tractability, the two-dimensional neural field model provides a modeling framework for studying localized propagating waves and their interactions.

Disclosures: Y. Qi: None. **P. Gong:** None.

Poster

094. Computation: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 94.03/BB48

Topic: G.06. Computation, Modeling, and Simulation

Support: Bernstein Award Udo Ernst, 01GQ1106

Title: Optimal feature integration in a critical, balanced network model

Authors: *N. TOMEN, U. ERNST;
Univ. of Bremen, Bremen, Germany

Abstract: Recent experimental and theoretical work established the hypothesis that cortical neurons operate close to a critical state which describes a phase transition from chaotic to ordered dynamics. This state is suggested to optimize several aspects of information processing. However, although critical dynamics have been demonstrated in recordings of spontaneously active cortical neurons, the link between criticality and active, realistic cortical computation remains largely unexplored. In our study we focus on visual feature integration as a prototypical and prominent example of cortical computation. In particular, we construct a network of integrate-and-fire neurons with balanced excitation and inhibition which performs contour integration on a visual stimulus. The network consists of orientation hypercolumns with biologically plausible connectivity and serves as a model for part of an early visual area (e.g. V1 or V2). In dependence on synaptic coupling strength, the network undergoes a transition from subcritical dynamics, over a critical state, to a highly synchronized regime. Near the critical regime, the network settles into a state of irregular spiking activity with intermittent avalanches of spikes which propagate preferentially over cortical columns which are processing the contour elements. Hence presenting a visual stimulus with a target figure dynamically organizes the network into two parts: one with critical dynamics, encoding the ensemble of features making up the figure (i.e., the contour), and one with subcritical dynamics, encoding the background elements. To quantify the network's computational capabilities, we consider a task where the contour has to be detected on the left or right part of the visual field. ROC analysis based on firing rate distributions reveals contour detection performances are maximized (~60%) near the critical state. In contrast, synchronized events allow for near perfect detection: Using coincidence detectors, we find maximum detection rates of 99.9% around the critical point. In short, we show that for different measures, contour detection performance is always maximized near or at the critical state. At the same time, our paradigm provides a unifying account for stylized features of cortical dynamics (i.e. high variability) and contour integration (i.e. high performance and robustness to noise) known from experimental studies. Lastly, our findings imply that in experimental studies where the cortex is engaged in active computation, avalanche analysis needs to be restricted to neurons processing behaviorally relevant features in order to find signatures of criticality.

Disclosures: **N. Tomen:** None. **U. Ernst:** None.

Poster

094. Computation: Other

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Title: Controllability of brain networks

Authors: ***S. GU**[1], F. PASQUALETTI[3], M. CIESLAK[4], S. T. GRAFTON[4], D. S. BASSETT[2]; [1]Applied Mathematics, [2]Dept. of Bioengineering, Univ. of Pennsylvania, PHILADELPHIA, PA; [3]Dept. of Mechanical Engin., Univ. of California, Riverside, Riverside, CA; [4]Dept. of Psychological and Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Cognitive function is driven by dynamic interactions between large-scale neural circuits or networks, enabling behavior. Fundamental principles constraining these dynamic network processes have remained elusive. Here we use recent theoretical advances in network control theory to offer a mechanistic explanation for how the brain moves between cognitive states, drawn from the network organization of white matter microstructure. Using diffusion spectrum imaging data acquired from 8 healthy subjects in triplicate, we construct structural brain networks between 234 brain regions (nodes) linked by the number of white matter streamlines connecting them. We employ a simplified noise-free linear discrete-time and time-invariant network model of neural dynamics in which the state of brain regions depends on the connectivity between them. Using this model, we compute the eigenvalues of the controllability Gramian for each brain region treated as a control node and demonstrate that structural brain networks representing the human brain are theoretically controllable, but extremely difficult to control in practice. We then determine how brain areas constrain or facilitate changes in brain state by computing three regional controllability diagnostics: the average, modal and boundary controllability. Our results indicate that densely connected areas are average controllers, theoretically expected to facilitate the movement of the brain to many easily-reachable states, and we show that these areas are preferentially located in the default mode system. Weakly

connected areas, predominantly located in cognitive control systems, are modal controllers, theoretically expected to facilitate the movement of the brain to difficult-to-reach states. Finally, areas located on the boundary between network communities, predominantly located in attentional control systems, are boundary controllers, theoretically expected to facilitate the integration or segregation of diverse cognitive systems. As a whole, this body of work suggests that structural network differences between the default mode, cognitive control, and attentional control systems dictate their distinct roles in controlling brain network function. More generally, our results support the view that macroscale structural design underlies basic cognitive control processes via the fundamental mechanism of network controllability.

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Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: Simons SCGB 26-7526-9012

ONR 26-1302-8712

Title: Exponentially-many states and robust error correction in Hopfield networks

Authors: *R. CHAUDHURI, I. FIETE;
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Abstract: Noise is ubiquitous in the brain, limiting how networks of neurons represent and propagate information. Consequently, the brain must encode signals redundantly and recover them in the presence of interference and noise. A fundamental question for neural computation asks how well neurons can do this: how many states can networks of neurons store and subsequently retrieve in the presence of noise? Current models yield either weak (i.e., sub-exponential) increases in representational capacity with network size or exhibit poor robustness to noise. The grid cell system in the entorhinal cortex has been shown to represent space with a capacity that scales exponentially with the number of cells and can in principle be robust to noise, but only with an appropriate decoder whose complexity is not yet well-characterized. We show that undirected graphs with Hopfield dynamics can have exponentially-many stable states

and, moreover, that errors in a finite fraction of nodes will be corrected by the dynamics, so that these states can be robustly recovered in the presence of noise. We construct these networks using sparse, bipartite expander graphs, which have previously been used to create novel, easily-decodable error-correcting codes. Such architectures combine sparse, weak constraints near-optimally to allow neurons to encode large numbers of states and to correct errors using simple, local dynamics. From a neural perspective these networks have a number of appealing features: they are sparse, can be constructed randomly, and connection strengths take values in a narrow range. Our results demonstrate that the structures of certain important error-correcting codes, wherein sparse constraints produce high-dimensional systems with large capacity and robustness, might apply to neural architectures.

Disclosures: **R. Chaudhuri:** None. **I. Fiete:** None.

Poster

094. Computation: Other

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Program#/Poster#: 94.06/BB51

Topic: G.06. Computation, Modeling, and Simulation

Title: How distinct is computational complexity from Shannon information?

Authors: *A. R. CASTI;
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Abstract: The complexity of a data set is a formal measure of symbolic structure that, like the better known Shannon Information, is measured in bits. In neuroscience, the Shannon Information has long been used to quantify a neuron's ability to encode and transmit information about a distribution of external stimuli. By comparison, complexity measures of neuronal output, such as spike trains, have received scant attention. Among other reasons, there appears to be no agreement on the best measure of neuronal complexity because most measures of it do not lend themselves easily to physiological interpretation. The best known complexity measure - the Kolmogorov Complexity - scales in proportion to the level of noise in the neuronal response, and thus may give a completely misleading picture of the underlying biophysics and neuronal circuitry that produced the response, and for what purpose. Further, because both the Shannon Information and complexity are intimately related to entropy, there is confusion regarding how distinct these measures really are. The primary aim of this work is to shed some light on this distinction for experimentally recorded and model generated spike trains of relay neurons in the visual thalamus. In this study, we use a physiologically interpretable complexity measure

introduced by Haslinger et al. (2010) - the computational complexity - that is derived from optimal Hidden Markov Models (Causal State Models) fit to spike train data. This approach determines the minimal number of "causal states" required to reproduce the statistical structure of the spike train, and distinguishes noise from intrinsic computational structure by defining the complexity as the entropy associated with causal state visitations in the data. We compare this complexity measure with the Shannon information of spike trains produced by a versatile model of relay neurons in the visual thalamus (Lateral Geniculate Nucleus), which includes calcium channels and feedforward/feedback inhibition to ensure a rich set of intrinsic causal states. We investigated the interplay between excitation and inhibition under a range of stimuli and model parameters, and asked whether parameters can be tuned to produce spike trains that are highly complex but have low Shannon information, and vice versa, or whether complexity and information are highly correlated. We conclude that computational complexity and Shannon Information are capable of generating distinct insights into neuronal spiking.

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Poster

094. Computation: Other

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Program#/Poster#: 94.07/BB52

Topic: G.06. Computation, Modeling, and Simulation

Title: Maintaining balance in networks with heterogeneous degree distributions

Authors: *R. PYLE[1], R. ROSENBAUM[2];
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Abstract: The balanced network modeling paradigm captures several features of cortical networks: asynchronous and irregular spiking activity, dense recurrent connectivity and a cancellation between strong excitatory and inhibitory synaptic currents [1]. Balanced networks are often modeled using unstructured, Erdős-Rényi style random graphs, where the probability of connection between any two neurons depends only the neuron type. However, experimental data reveal a more intricate structure with neurons exhibiting large variability of in-degrees, out-degrees and synaptic weights [2]. We explore the notion of balanced excitation and inhibition in networks with heterogeneous connectivity structure. It was recently shown that power-law degree distributions cause a loss of balance [3]. We use a heterogeneous mean-field theory [4] to show that this conclusion relies critically on an assumption that in- and out-degrees are uncorrelated. Introducing correlations between a neuron's in- and out-degrees can recover the

balanced state, but leads to the surprising conclusion that neurons with a high in or out-degree have a lower firing rate. Our mean-field analysis is supplemented with simulations of biologically realistic neuron models. References 1. Van Vreeswijk, C., & Sompolinsky, H. (1996). Chaos in neuronal networks with balanced excitatory and inhibitory activity. Science (New York, N.Y.), 274(5293), 1724-6. 2. Song, S., Sjöström, P. J., Reigl, M., Nelson, S., & Chklovskii, D. B. (2005). Highly nonrandom features of synaptic connectivity in local cortical circuits. PLoS Biology, 3(3), e68. 3. I.D. Landau, R. Egger, M. Oberlaender, H. Sompoloinsky. The relationship between microcircuit structure and dynamics in the rat barrel cortex. Program No. 535.02. 2014 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2014. Online. 4. di Volo, Matteo, et al. "Heterogeneous mean field for neural networks with short-term plasticity." Physical Review E 90.2 (2014): 022811.

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Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: Career Award at the Scientific Interface from Burroughs Wellcome Fund

Title: Plasticity-induced sensitization in recurrent E-I networks

Authors: *G. KUMAR[1], S. CHING[2];
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Abstract: The effects of short- and long-term plasticity on information processing in sensory networks remains a fundamental question in sensory neuroscience. Here, we use a combination of computational modeling and systems-level analysis to study how long-term plasticity shapes the sensitivity of E-I network to afferent excitation. Specifically, we implement a rate-based recurrent neuronal network, endowed with a Bienenstock, Cooper, Munro (BCM) synaptic adaptation rule. The network is impinged upon by high dimensional afferent inputs. At each moment in time, we perform a reachability analysis, based on methods derived from control theory, to study the geometry of the space of possible (i.e., inducible) output trajectories (i.e., network activity as a function of time). Our overall goal is to characterize how this geometry

changes, via the plasticity, as a function of repetitive stimuli delivered to the network. Using both numerical simulation and exact analysis, we found that recurrent E-I networks demonstrated highly specific spatial sensitization with respect to ongoing afferent excitation. In particular, we found that the space of reachable trajectories (neuronal activation patterns) contracts in the direction associated with the ongoing stimulus, while simultaneously expanding in orthogonal directions. In other words, the network de-sensitizes to the current stimulus, while at the same time, sensitizes to potential competing/distracting stimuli. Importantly, this sensitization is mediated strictly via the intrinsic dynamics and not via attenuation in the gain from the extrinsic input onto the network. Thus, our findings suggest that long-term plasticity enables a network to favor stimuli that are different/novel from ongoing activity. Further, our results show that such a property is innate to recurrent E-I networks, suggesting that such networks intrinsically support enhanced discriminability of novel inputs.

Disclosures: **G. Kumar:** None. **S. Ching:** None.

Poster

094. Computation: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 94.09/BB54

Topic: G.06. Computation, Modeling, and Simulation

Support: ARC Centre Grant CE140100007

Title: A modeling study of the dynamics of memory retrieval

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Science, Sch. of Physics, Complex Syst. Group, Univ. of Sydney, Sydney, Australia

Abstract: The capability of human memory system to retrieve task relevant items is remarkable. Evidence has been accumulated to indicate that memory retrieval process has certain regularities. For instance, in category fluency test, a paradigm used to study human memory retrieval (e.g., name as many animals as possible in one minute), semantically closely related items tend to be retrieved consecutively, forming a cluster, and the retrieval process switches between such semantic clusters. However, the frequency of such switches has been shown to be significantly (from 40% to 55%) lower in patients with Alzheimer's disease or the Parkinson's disease than that in healthy subjects. Despite the importance of this kind of switching process in memory retrieval, its dynamic nature and underlying neurophysiological mechanisms remain largely unknown. We develop a network model consisting of conductance-based integrate-and-fire

neurons. Excitatory neurons form several densely connected modules, each of which can be used to store a memory. A hierarchical structure is further imposed upon the inter-module wiring probabilities, representing similar structure found in both neuron coupling topology and semantic networks. We find that, when the excitation and inhibition is roughly balanced, the system spontaneously activates one neuron module after another; in this process, consecutive activations are separated by almost quiescent states. This dynamic process can be regarded as a free memory retrieval process. We further demonstrate that the semantic dependence of the free memory retrieval process and the heavy-tailed distribution of the inter-retrieval intervals, as found in psychological experiments, can be reproduced in our model. However, when the E-I balance or the hierarchical structure is broken, there is severe impairment in the memory retrieval performance. At the neurophysiological level, this result is consistent with the experimental observation that there exists an elevated excitation-inhibition ratio and a decrease of neural connections in patients with Alzheimer's disease or Parkinson's disease; at the behavioral level, this result is consistent with the fact that a significant deficit in memory retrieval is a typical syndrome of these patients.

Disclosures: **Y. Gu:** None. **P. Gong:** None.

Poster

094. Computation: Other

Location: Hall A

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Program#/Poster#: 94.10/BB55

Topic: G.06. Computation, Modeling, and Simulation

Title: High dimensional firing rate dynamics in spatially extended asynchronous networks

Authors: ***R. ROSENBAUM**;
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Abstract: Cortical circuits exhibit asynchronous, irregular dynamics which are often modeled computationally using networks of randomly connected integrate-and-fire neurons [1]. Such models produce complex microscopic dynamics – at the level of individual spikes – but these dynamics are largely unreliable from trial to trial. Macroscopic dynamics – at the level of firing rates – are highly reliable, but extremely simple, as they merely track feedforward input. Non-trivial neural computations require reliable responses with rich dynamical structure [2]. We propose that introducing spatial structure to asynchronous, irregular networks can impart rich and reliable firing rate dynamics. We combine spatial neural field theory with linear response theory to analyze the stability of homogeneous firing rates in spatially extended networks of

spiking neuron models. In doing so, we discover the presence of spatio-temporal pattern forming bifurcations that produce complex firing rate dynamics. We show that these bifurcations cannot be reproduced in simpler "firing rate" models that are often used to study spatially extended networks. Moreover, the dynamics produced by the bifurcations are equally high-dimensional, but more reliable than those produced by unstructured random networks with strong synaptic coupling [3]. Our results suggest that intrinsic spatio-temporal dynamics is a realistic model for the rich dynamics underlying non-trivial neural computations [2]. [1] Brunel, N. (2000). Dynamics of sparsely connected networks of excitatory and inhibitory spiking neurons. Journal of Computational Neuroscience, 8(3), 183–208. [2] Sussillo, D., & Abbott, L. F. (2009). Generating coherent patterns of activity from chaotic neural networks. Neuron, 63(4), 544–57. [3] Ostojic, S. (2014). Two types of asynchronous activity in networks of excitatory and inhibitory spiking neurons. Nature Neuroscience, 17(4), 594–600.

Disclosures: R. Rosenbaum: None.

Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: Doctoral Grant from Fundació "la Caixa"

Project Grant from the Spanish Ministry of Economics and Competitiveness BFU2012-33413

Title: Different models of network connectivity can explain “non-random” features of cortical microcircuits

Authors: *M. VEGUÉ, A. ROXIN;
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Abstract: Network topology shapes the ability of neural networks to store and transmit information. In neuronal network models the topology is represented by a directed graph, which is a set of vertices (the neurons) and a set of directed edges that connect them in a precise manner (the synapses). One of the challenges of network modeling is, therefore, the construction of random graphs that reflect both the variability and the main structural properties of real neural networks. Erdös-Rényi (ER) graphs, defined by a single parameter p which determines the

probability of any directed edge, are among the simplest random models and have been used in a broad range of theoretical studies. Some experimental studies suggest, however, that cortical microcircuits are not well represented by ER models [1], [2]. Here we discuss several alternative classes of network models for fitting the available data: 1 - networks with distinct neuronal clusters, 2 - networks with spatially decaying connectivity, and 3 - networks with broad, correlated in-degree and out-degree distributions. We find that all three classes of networks fit the available data well, including doublet and triple motifs [1] and the increase in the likelihood of connectivity between neurons as a function of common neighbors [2]. Interestingly, networks of the third class, namely, with broad, correlated degrees, do not include any clustering, indicating that one should use caution in drawing inferences about such higher order structure from lower order statistics. Finally, we study the dynamics of networks of spiking neurons for each of the three classes of network connectivity. Specifically, we consider the networks in the so-called fluctuation-driven in which recurrent excitatory and inhibitory currents are large and balanced, leading to spiking statistics similar to that seen *in vivo*: e.g. CV of ISI near 1, low mean firing rate, broad firing rate distributions. In this regime we look for dynamic signatures of each network class which could be used to aid in distinguishing the underlying network structure. We find that the network response to transient stimuli can temporarily break the balance of currents in the stationary regime, thereby generating dynamics which is strongly shaped by the underlying network structure. [1] Song et al. PloS Biol. 2005. [2] Perin et al. PNAS 2011.

Disclosures: **M. Vegué:** None. **A. Roxin:** None.

Poster

094. Computation: Other

Location: Hall A

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Program#/Poster#: 94.12/BB57

Topic: G.06. Computation, Modeling, and Simulation

Title: Electrode-position- and equivalent-current-dipole-source-localization-based brain functional connectivity networks using scalp-recorded EEGs: A comparison of Alzheimer's disease patients and healthy subjects

Authors: R. URATA[1], *T. YAMAZAKI[2], Y. KUROIWA[3];
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Abstract: Objective: We propose electrode-position- and equivalent-current-dipole-source-localization (EDCL)-based brain functional connectivity networks (BFCNs), where the latter will

enable us to compare with the previous BFCN studies using fMRI. Methods: Nineteen-channel EEGs were recorded during an eye-closed resting state in two female Alzheimer's disease (AD) patients and five healthy subjects. Correlations between any two nodes were calculated by synchronization likelihood (SL). Results: Among the network parameters, there were remarkably significant differences in the small-worldness and the betweeness centrality between the AD patients and healthy subjects for gamma band and alpha, beta and gamma ones, respectively. Considerations: Electrode positions and the brain regions, obtained by the previous fMRI-based BFCN, with the high betweenness centrality were considerably similar, which was confirmed also by our EDCL-based one. Moreover, effects of medications on the AD patients will be visualized and examined by the present BFCNs.

Disclosures: **R. Urata:** None. **T. Yamazaki:** None. **Y. Kuroiwa:** None.

Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: Sandia National Laboratories Laboratory Directed Research and Development

James S McDonnell Foundation

Title: Quantifying neural information content: a case study of the impact of hippocampal adult neurogenesis through computational modeling

Authors: ***C. M. VINEYARD**, S. J. VERZI, C. D. JAMES, J. B. AIMONE;
Sandia Natl. Labs., Albuquerque, NM

Abstract: There are multiple perspectives on hippocampal function. Focusing upon the effects of information encoding and transformation occurring in the hippocampal loop, here we show some insight provided by a less conventional interpretation inspired by machine learning principles. We hypothesize that the role of the hippocampus in information processing is to restructure and refine the multimodal associative encoding provided by the entorhinal cortex (EC) through a high dimensional adaptive transformation in dentate gyrus (DG) and subsequent compressive encoding by the CA3. This computational process is analogous to a Support Vector Machine (SVM). However, unlike conventional SVM theory, the existence of adult neurogenesis in DG confers an adaptive high dimensional projection which impacts the resulting CA3

encoding (or discriminant in a canonical SVM). To explore this hypothesis as well as the impact of adult neurogenesis, we have developed metrics and computational paradigms to quantify neural information content. Neural networks have an intrinsic information content encapsulated by neural firing behaviors and constituted by their neural encoding. Shannon entropy is a fundamental method to quantize the amount of information in a variety of sources such as communication channels. Many approaches have been devised to apply this sort of information measure to neurons with mixed success. However, doing so typically requires knowledge of the firing behavior probability distributions for the neurons of interest (whether modeled or recordings), and furthermore often is only applicable for single neurons and not ensembles. We have observed that conventional compression methods may help overcome some of the limiting factors of standard techniques and allows us to approximate information in neural data. To do so we have used compressibility as a measure of complexity in order to estimate entropy to quantitatively assess information content of neural ensembles. Using Lempel-Ziv compression we are able to assess the rate of generation of new patterns across a neural ensemble's firing activity over time to approximate the information content encoded by a neural circuit. As a specific case study, we have been investigating the effect of neural mixed coding schemes due to hippocampal adult neurogenesis.

Disclosures: **C.M. Vineyard:** A. Employment/Salary (full or part-time):; Sandia National Laboratories. **S.J. Verzi:** A. Employment/Salary (full or part-time):; Sandia National Laboratories. **C.D. James:** A. Employment/Salary (full or part-time):; Sandia National Laboratories. **J.B. Aimone:** A. Employment/Salary (full or part-time):; Sandia National Laboratories.

Poster

094. Computation: Other

Location: Hall A

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Program#/Poster#: 94.14/BB59

Topic: G.06. Computation, Modeling, and Simulation

Support: JSPS Grants-in-Aid KAKENHI 23240065

JSPS Grants-in-Aid KAKENHI 26560202

JSPS Grant-in-Aid for JSPS Fellows 26-8435

Title: An information-theoretical interpretation for neural modulation of spike-timing dependent plasticity: Towards cellular-based computational psychiatry

Authors: *T. ISOMURA[1], K. SAKAI[2], Y. SATO[2], K. KOTANI[3], Y. JIMBO[2];
[2]Dept. of Precision Engineering, Sch. of Engin., [3]Res. Ctr. for Advanced Sci. and Technol., [1]The Univ. of Tokyo, Tokyo, Japan

Abstract: Friston's free-energy hypothesis, a candidate unified theory for learning and memory in the brain, predicts that neurons, synapses, and neuromodulators work in a manner to minimize free energy. However, electrophysiological data elucidating the neural and synaptic bases for this theory are lacking. Here, we propose a novel theory linking the information-theoretical principle with the biological phenomenon of spike-timing dependent plasticity (STDP) modulated by neuromodulators, which we have termed mSTDP. We proposed that an mSTDP algorithm is derived from an enhanced version of Friston's free energy (an information theoretical function) and analytically and numerically showed that dopamine (DA) and noradrenaline (NA) modulate the accuracy of principal component analysis (PCA) performed using the mSTDP algorithm. Specifically, synaptic connection strengths (an N-dimensional vector) were established such that in the absence of DA/NA, the neural output represented the first principal component (PC1), which is the direction where the information represented by the neural output is maximized (Infomax learning). DA up-regulation affected all connections of a neuron with all synapses attaining similar amplitudes, while NA up-regulation markedly decreased the amplitude of almost all connections except only a few ones, in a winner-takes-all manner. Next, we found that, in the case of 2-dimensional input, increasing DA concentration resulted in the shift of the preferred direction of the neuron to $\theta = \pi/4$, which indicates that both synaptic connections attained a similar amplitude, while increasing NA concentration tuned the preferred direction to $\theta = 0$ or $\pi/2$. Therefore, if DA or NA concentrations increase, neural networks shift to perform pattern completion or separation, respectively. These results are consistent with electrophysiological findings and validate the free-energy principle and mSTDP theory. Moreover, our algorithm can potentially be applied for computational psychiatry. Our results suggest that when DA concentrations are abnormal, neuron tends to regard their input as more highly correlated than it actually is and neural networks fail to represent the external world accurately; this can be used to design a computational model of hallucination and delusion, which are common positive symptoms of schizophrenia. On the other hand, when NA concentrations are abnormal, neurons tend to increase a specific synaptic connection and fail to perform appropriate processing; this finding can aid in building a computational model of NA contribution in memory triage and post-traumatic stress disorder.

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Poster

094. Computation: Other

Location: Hall A

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Program#/Poster#: 94.15/BB60

Topic: G.06. Computation, Modeling, and Simulation

Title: Measuring causality in simulations of large scale brain networks

Authors: *M. MANNINO[1], S. BRESSLER[2];
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Abstract: Inferring causal relations between the nodes of large-scale brain networks can reveal network topology, connectivity, and function, and help uncover the neurobiological basis of cognition. Yet appropriate methodologies for measuring dynamic causality in brain networks remain varied and elusive. The application of directed functional connectivity analysis to simulated fMRI BOLD data can recover neural connectivity patterns and validate inferences from causality analysis of veridical fMRI BOLD data (Tang et al, 2012). However, simulated data has not previously been widely used because it has failed to capture important underlying neural mechanisms (Bressler et al, 2008). Here, we perform computational simulation and mathematical analysis on a neuroinformatics platform called The Virtual Brain (TVB) that simulates biologically realistic neural population data generated by a large-scale distributed brain network model. TVB is unique in the field of neural simulation in that it is organized at the mesoscopic level of large-scale brain networks with nodes that are neural populations. TVB provides a framework for evaluating various methodologies on biologically plausible simulated data (Wang et al, 2014). The overall objective of this project is to use a nonlinear dynamical system, specifically a neural mass model to generate time series data of a local field potential (LFP) signal, and use linear autoregressive modeling to analyze the data, thereby inferring causal relations in large-scale brain networks. Data are generated from two coupled oscillatory neural masses, with the Stefanescu-Jirsa 3D model in the TVB simulator governing the intrinsic dynamics of each population. Trials vary parameter values, including conduction velocities and model parameters that determine excitatory and inhibitory coupling. This approach allows comparison of Granger causal analysis of simulated and veridical BOLD time series to determine the directionality of influence in large-scale brain networks.

Disclosures: **M. Mannino:** None. **S. Bressler:** None.

Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: Israel Science Foundation Grant No. 1733/13

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Gatsby Charitable Foundation

Title: Continuous parameter working memory in a balanced chaotic neural network

Authors: *N. SHAHAM[1], Y. BURAK[2,1];
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Abstract: There has been considerable theoretical and experimental interest in the storage in working memory of continuous parameters, such as direction, color, or frequency. One of the main theoretical models of working memory of continuous parameters is local circuits in the brain that exhibit continuous attractor dynamics, where different values of a stimulus can be represented by different locations along a continuum of steady states. However, it has been unclear whether this theoretical idea is compatible with another proposal for the architecture of cortical circuits - the balanced network (van Vreeswijk and Sompolinsky, Science 1996, Neur Comp 1998). In previous work (Mato and Hansel, J. Neurosci 2013; Goldman and Lim, Nat. Neurosci 2013, J. Neurosci 2014) slow dynamics within a balanced network was achieved using complementary mechanisms, such as multiple synaptic time scales or short term plasticity. In this work we study a network with random connectivity which generates a balanced state. Using mutual inhibition between two balanced populations, we find an architecture for which the network can sustain slow dynamics in a certain direction in the mean-activity space. The persistence is achieved without using short term plasticity or multiple synaptic time scales. The slow dynamics make this balanced network an appropriate candidate for the storage of working memory. We find that the chaotic dynamics of neural activity in the balanced network drives diffusive motion along the attractor, similar to the dynamics of networks in which noise arises from intrinsic neural or synaptic mechanisms (Burak and Fiete, PNAS 2012). In addition to the diffusive motion, the network can exhibit systematic motion due to mistuning of the network parameters, and the overall dynamics along the attractor follows similar statistics to an Ornstein-Uhlenbeck process. Using analytical and numerical analysis, we show that the diffusion coefficient along the attractor is inversely proportional to the network size. Thus, the persistence of the network can be improved by increasing the number of neurons. In practice, ~10^5 neurons are sufficient in our model (with proper tuning of the synaptic weights) to achieve persistence times of several seconds, larger by several orders of magnitude than the single neuron time scale.

Disclosures: **N. Shaham:** None. **Y. Burak:** None.

Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: FP7-PEOPLE-2012 Maria Skłodowska-Curie International Outgoing Fellowship (Project 331486 "Neuron-Astro-Nets")

Title: Persistent delay activity in a neuron-glia network model

Authors: *M. DE PITTÀ[1,3], H. BERRY[3], N. BRUNEL[1,2];
[1]Dept. of Neurobio., [2]Dept. of Statistics, The Univ. of Chicago, Chicago, IL; [3]EPI Beagle, INRIA Rhône-Alpes, Villeurbanne cedex, France

Abstract: In primates performing delayed response tasks, the persistent increase of neural firing during the delay period is regarded as the neuronal hallmark of working memory that is the transient maintenance and manipulation of goal-related information for forthcoming actions. Several mechanisms have been suggested for the emergence of persistent delay activity, but it remains unknown whether it stems from intrinsic properties of neurons, the nature of their synaptic connections, or both. In recent years, astrocytes, the main type of glial cells in the brain, have been suggested as potential active players in neural function due to their ability to regulate synaptic transmission by releasing gliotransmitters, like glutamate or ATP, in an activity-dependent fashion. Because available models of persistent activity do not take into account astrocytes, we set to investigate the emergence of persistent delay activity in a spiking model of cortical neuron-glia networks focusing on one putative mechanism: the activity-dependent regulation of neurotransmitter release at excitatory synapses by gliotransmitters. Mean field analysis of the model suggests that long-lasting gliotransmitter-mediated increases of synaptic glutamate release could be responsible for the appearance of a persistent mode of high synaptic release that coexists with that of low synaptic release in the absence of astrocytic modulation. In this fashion, the transient increase of neural firing upon presentation of a stimulatory cue could trigger astrocytic modulation and switch synaptic release from low to high. In turn, high synaptic glutamate release could further promote astrocyte activation and modulation of synaptic transmission ultimately resulting in increased postsynaptic neural firing during the delay period. In this fashion, the increase in neural firing becomes persistent as long as gliotransmitter release from the astrocyte occurs. This mechanism is robust and could be observed both in single neurons as well as in a classical balanced cortical network model. Taken together, these results suggest a novel astrocyte-based mechanism for persistent activity, and provide experimentally

testable hypotheses for the possible involvement of astrocytes in cognitive tasks related to working memory.

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Poster

094. Computation: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 94.18/BB63

Topic: G.06. Computation, Modeling, and Simulation

Title: Interactions between neural circuits that mediate social and nonsocial behaviors

Authors: *J. HURTADO[1], D. F. RAMIREZ[1], T. J. SEJNOWSKI[2];
[1]Univ. Autonoma De Occidente, Cali, Colombia; [2]CNL, The Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Understanding circuit-level mechanisms mediating aggression behaviors as well as how aggression, mating, and the associated synaptic circuits relate to each other remains poorly understood. In recent researches, optogenetic manipulations in certain brain nuclei in mice have shown the relationship of aggression circuits to those mediating opponent social behaviors, such as mating, and a nonsocial behavior as self-grooming [1]. Despite the new findings, relatively little is known about the circuit-level function of the neurons that control social behaviors. In order to give some insights and to continue providing approaches for explaining the mechanisms and processes that might control social behaviors, we have proposed a biologically inspired neural network model. First, we propose possible interactions among self-grooming, mating, and fighting circuits. Our model includes populations of neurons in the amygdala and hypothalamus as well as amygdalar-hypothalamic pathways to promote either self-grooming, mating, or fighting and pathways to suppress these behaviors. Second, the mathematical model is based on attractor networks with dynamical stability, which allows us to provide a framework for understanding the rich and complex neural activity patterns generated in recurrent networks. Finally, the model is examined with bifurcation analysis and computer simulations. The results demonstrate that the model exhibits stable steady states and thresholds for steady state transitions corresponding to some experimentally observed behaviors, such as aggression control. [1] Hong W, Kim DW, Anderson DJ: Antagonistic Control of Social versus Repetitive Self-Grooming Behaviors by Separable Amygdala Neuronal Subsets, Cell 2014, 158:1348-1361.

Disclosures: **J. Hurtado:** None. **D.F. Ramirez:** None. **T.J. Sejnowski:** None.

Poster

094. Computation: Other

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Program#/Poster#: 94.19/BB64

Topic: G.06. Computation, Modeling, and Simulation

Support: NSF Grant IOS 1054914

Title: Low-dimensional attractor of neural activity from optogenetic data

Authors: *P. G. LYNN[1], L. EVANS[2], S. OPRISAN[3], T. TOMPA[4], A. LAVIN[4];
[1]Col. of Charleston, New Smyrna Beach, FL; [2]Psychology, [3]Physics and Astronomy, Col. of Charleston, Charleston, SC; [4]Dept. of Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: We used optogenetic mice to investigate the response of the medial prefrontal cortex (mPFC) local network to light stimuli delivered by a 473 nm laser through a fiber optics. Local field potential (LFP) recordings obtained with an optrode were band-pass filtered online between 0.1 and 130 Hz. The entire experimental protocol consisted of two successive two-second long recordings in response to (1) a 40 Hz, 10-pulses train, that lasted 250 ms with 10 ms pulse duration followed by a 15 ms break, and (2) a single pulse with 10 ms duration. We analyzed the response of the network to a single 10 ms duration light pulse using delay embedding method. We found that the dynamics could be reconstructed in a three-dimensional space. Our results open the possibility of designing a low-dimensional model for optical stimulation of the local network.

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Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: Dr. Ralph and Marian Falk Medical Research Trust Fund

Title: Robustness of pair-correlated network activity in the presence of noise

Authors: *J. NEUMAN[1], W. VAN DRONGELEN[2], J. COWAN[3];
[1]Physics, [2]Pediatrics, [3]Mathematics, Univ. of Chicago, Chicago, IL

Abstract: One of the most important unsolved problems in neuroscience is the question of the nature of correlated activity patterns. *In vivo* experiments have shown that activity in neighboring cortical areas is more correlated and extends over a greater distance when no external stimulus is present (Nauhaus et al. 2009). Because random fluctuations are omnipresent across the whole nervous system and come in many forms, an understanding of the effect each source of noise has on spontaneous and stimulated modes is a significant topic to examine. In this modeling study, we investigate the effects of various noise types and strengths, as measured by the signal-to-noise ratio, on correlated activity between coupled populations of neurons. Our results show that the pair-correlations between a reference area and its neighboring patches of cortex in both spontaneous and driven regimes are qualitatively very robust to many different sources of noise. In particular, we use a stochastic single neuron Markov model to recreate intrinsic multiplicative neuronal noise and a network model to simulate additive synaptic noise. Both models are consistent with the experimental findings mentioned above when the network size is varied as well as the source of synaptic noise. Specifically, we have implemented noisy inputs, outputs, neuronal thresholds, and connectivity strengths for non-pathological biologically plausible signal-to-noise ratios ranging from 5-15 and found robustness in the pair correlation coefficient. This result has many potential implications. First, it says external stimuli play a significant role in brain dynamics. While this seems obvious, the comparison in strengths of peripheral drives to random variations is not trivial. Next, these findings suggest that many different sources of noise can be lumped together when studying behavior at large cortical scales. The specific noise variety is not a major consideration. Lastly, it says resting brain activity might be very stable to random fluctuations. This is particularly useful in helping the brain optimize its computational power.

Disclosures: J. Neuman: None. **W. van Drongelen:** None. **J. Cowan:** None.

Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH Pioneer DP1EY024503

Title: Calcium imaging reveals multiple conduction systems in hydra

Authors: *C. DUPRE, R. YUSTE;
Columbia Univ., New York, NY

Abstract: Hydra is representative of the most primitive nervous systems. Yet, it turns out that it does not function like its structure (a nerve net) would suggest and there might be more to learn about neurons in hydra than previously thought. This cnidarian offers a convenient preparation for calcium imaging, especially because it is possible to image the entire animal simultaneously. Our experiments using such technique revealed multiple groups of neurons (or conduction systems) that are anatomically distinct and that fire simultaneously, together with a series of neurons that seem to fire independently. Accordingly, we are interested in dissecting the nervous system of hydra and answering the following questions: How many different circuits are there in hydra? What type of computation does each of these circuits do, and to what extent are these circuits dependent on each other? Answering these questions will help understand the properties of the most primitive nervous systems and how they produce the appropriate behavioral response to a given situation.

Disclosures: C. Dupre: None. **R. Yuste:** None.

Poster

094. Computation: Other

Location: Hall A

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Program#/Poster#: 94.22/BB67

Topic: G.06. Computation, Modeling, and Simulation

Title: Stability of strongly coupled inhibitory-excitatory networks with realistic synaptic dynamics

Authors: *K. DIPIETRO;
Applied Computat. Mathematics and Statistics, Univ. of Notre Dame, Notre Dame, IN

Abstract: Cortical circuits are believed to operate in a balanced or inhibitory-stabilized regime in which strong recurrent excitation is matched by strong inhibition [1,2]. Such networks are often modeled computationally using two-dimensional mean field equations such as the Wilson-Cowan model [1,3] or by using balanced networks of spiking units [2]. Both of these modeling approaches rely on the implicit assumption that inhibition is faster than excitation. The validity

of this assumption is not immediately clear. While the membrane dynamics of inhibitory interneurons are often faster than those of excitatory pyramidal neurons, GABAergic synaptic kinetics are generally slower than AMPA-mediated glutamatergic synaptic kinetics. We systematically address the question of how cortical networks can maintain a stable excitatory-inhibitory balance when inhibitory synaptic kinetics are slower than excitatory synaptic kinetics. We begin by extending the Wilson-Cowan model to a four-dimensional system that separates synaptic and neuronal timescales [4]. From this extended mean-field model, we derive concise conditions on the stability of excitatory-inhibitory balance in the asymptotic limit of strong coupling. We then verify and generalize the qualitative findings from the mean-field model using a numerical stability analysis of integrate-and-fire networks with biologically realistic parameters [4]. [1] Ozeki, H., Finn, I. M., Schaffer, E. S., Miller, K. D., & Ferster, D. (2009). Inhibitory stabilization of the cortical network underlies visual surround suppression. *Neuron*, 62(4), 578–92. [2] Van Vreeswijk, C., & Sompolinsky, H. (1996). Chaos in neuronal networks with balanced excitatory and inhibitory activity. *Science*, 274(5293), 1724–6. [3] Wilson, H. R., & Cowan, J. D. (1972). Excitatory and inhibitory interactions in localized populations of model neurons. *Biophysical journal*, *12*(1), 1. [4] Ledoux, E., & Brunel, N. (2011). Dynamics of networks of excitatory and inhibitory neurons in response to time-dependent inputs. *Frontiers in Computational Neuroscience*, 5(May), 25.

Disclosures: K. Dipietro: None.

Poster

094. Computation: Other

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 94.23/BB68

Topic: G.06. Computation, Modeling, and Simulation

Title: Finding the right firing rate and growing the right synapses: maximizing the emergence of characteristic neural circuit features through maximal self-organization

Authors: *Z. TOSI;
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Abstract: The quantity and quality of our tools for probing the structural and behavioral features of neural circuits have rapidly improved in recent years, putting ourselves in a situation where data pertaining to these features is in abundance but models accounting for said features are somewhat lacking. Indeed, many models can account for at least some subset of these features,

but models which account for them more broadly are few and far between. Furthermore, the majority models that do exist tend to have one or more observed features parameterized into the network a priori, thus eliminating any possible insight as to how the parameterized features may come about. This has brought the demand for self-organizing features in neural circuit models to a much needed all-time high. Building upon the work of Jochen Treisch and colleagues' SORN (Self Organizing Recurrent Network) model (2009), the following (distinct) work sets out to self organize in a way which minimizes parameterized features and maximizes emergent features consistent with observed behavior in living neural circuits. By combining a form of intrinsic plasticity which promotes the emergence of log-normally distributed firing rates, with homeostatic plasticity, continuously applied additive spike timing dependent plasticity (STDP), inhibitory-STDP, resource-constrained synaptic potentiation, and STDP guided synaptic growth and pruning, the following network accomplishes that goal. The resulting network produces log-normally distributed firing rate and synaptic efficacy distributions, but also captures many aspects of the connectivity structure found in living neural circuits. These include a highly non-random correlation among incoming connections to neurons, heavy-tailed degree distributions, qualitatively similar node versatility distributions, and the emergence of rich-clubs in the network. Additionally, tests of the resulting network's computational power in terms of its kernel quality (separation ability) and VC-Dimension (generalization ability) suggest a high degree of computational ability. To the author's knowledge this is the first time a single network model has been able to account for all these features without explicitly coding them into the model, and would offer researchers the opportunity to "grow" highly realistic generic artificial microcircuits, as well as offer predictions as to how these many features may emerge in living organisms.

Disclosures: **Z. Tosi:** A. Employment/Salary (full or part-time):; Indiana University. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); National Science Foundation.

Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: NSF Grant IOS 1054914

MAYS Grant College of Charleston

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HHMI Fellowship College of Charleston

Title: Phase resetting induced by concurrent stimuli

Authors: *S. OPRISAN[1], K. M. VOLLMER[2], D. AUSTIN[3], L. EVANS[4];
[1]Physics & Astronomy, [2]Biol., [3]Physics and Astronomy, [4]Psychology, Col. of Charleston, Charleston, SC

Abstract: The PRC tabulates the transient change in the firing period of a neuron due to external perturbations, such as presynaptic stimuli. The PRC is the input-output transfer function of a neuron and is useful in predicting the phase-locked modes of neural networks. To measure the PRC, a single pulse is applied at different phases during the cycle. The open loop pulse applied to an isolated neuron mimics as closely as possible the presynaptic input received by the respective neuron when it is part of the neural network (closed loop). The single pulse produces transient changes of the firing period over multiple cycles, which are measured by PRCs of different orders. We focused only on the first order PRC that describes the transient change in the length of the cycle containing the pulse. Since neurons receive more than one presynaptic input per cycle, we generalized the single pulse PRC to the more realistic case of neural oscillators receiving two or more inputs per cycle. We used a conductance-based model neuron to estimate experimentally the two-stimuli PRC and compare the results against our mathematical prediction based on the assumption of instantaneous recurrent stimulation. Within the limits of the recurrent stimulation assumptions, we found that the newly introduced prediction for the two-stimulus PRC matches experimental measurements. Our new results open the possibility of a more realistic approach to predicting phase locked modes in neural networks, such as the synchronous activity of large networks during epileptic seizures.

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Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: Helmholtz Association: portfolio theme SMHB and Young Investigator's Group VH-NG-1028

EU Grants 269921 (BrainScaleS)

604102 (Human Brain Project)

Title: The anatomical origin of locally generated and induced oscillations in a model of the cortical microcircuit

Authors: *H. BOS[1], J. SCHÜCKER[1], M. DIESMANN[1,2,3], M. HELIAS[1];
[1]Inst. of Neurosci. and Med. INM-6, Inst. for Advanced Simulation IAS-6, Jülich Res. Ctr. and JARA, Jülich, Germany; [2]Dept. of Psychiatry, Psychotherapy and Psychosomatics, Med. Fac., [3]Dept. of Physics, Fac. 1, RWTH Aachen Univ., Aachen, Germany

Abstract: Fast oscillations of the population firing rate in the high gamma range (50-200 Hz), as well as slow firing rate fluctuations are ubiquitous in cortical recordings and have been hypothesized to be generated locally. Utilizing the recently established multi-layered spiking neural network model of a cortical microcircuit and our mean-field theoretical framework, we address the question of the anatomical origin of the observed oscillations by analyzing the spectra generated in the resting condition as well as under the application of constant and oscillatory input. Deriving the theoretical framework we perform a two-step reduction allowing for an incremental validation of first the prediction of the population firing rates and second the prediction of the population rate spectra. Building on previous work that derived the mapping of populations of leaky integrate-and-fire model neurons to a linear rate model using the response function of populations of neurons connected by exponentially decaying synapses, the mean-field framework is applicable to arbitrary circuitries set in the asynchronous irregular regime. In the resting condition the neurons in the model fire irregularly, displaying little synchrony on the population level. Increasing the external input to the excitatory population in layer 5 elicits slow rate fluctuations, reflected as elevated slow frequency components in the population rate spectra. Strengthening the input to the superficial layers triggers population oscillations in the gamma range, while individual neurons preserve low rates close to irregularity. We derive a sensitivity measure determining the anatomical connections within the circuit crucial for the generation of the peaks visible in the power spectra as well as their impact on the peak frequencies and amplitudes. We identify a sub-circuit located in layer 2/3 and 4 constituting the basis of the high frequency oscillations, while connections within and onto layer 5 determine the existence and strength of slow rate fluctuations. Since the sensitivity measure is derived from the mean-field theory we can analyze the robustness of these findings under changes of parameters in the neuron and synapse model and conclude on the actual contributions of the anatomical connections given their embedding in the full circuit. Exploiting the mean-field framework we generate predictions for the population-specific changes in the power spectra under various stimulation protocols. Inspired by published experimental studies, we analyze the responses of the circuit to oscillatory input and formulate predictions for the susceptibility of individual populations to specific frequencies.

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Poster

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Program#/Poster#: 94.26/BB71

Topic: G.06. Computation, Modeling, and Simulation

Support: A*STAR JCO grant #1335h00098

Title: Sequence learning using pre-configured cell assemblies

Authors: *Y. LEOW, S. G. LALLÉE, C. Y. TAN;
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Abstract: Sequential events are inherent to ongoing experience, and the ability to represent coherent episodes while preserving sequential order is critical for planning future actions. Hippocampal neural assemblies have been shown to be activated in a sequential order reflecting behavioural content at a faster timescale during offline (eg. awake immobility & sleep) periods. Such replay sequences have been observed in both forward and reverse order under different behavioural states. Replay sequences provide a window to explore how the brain represents and preserves contiguity of ongoing experience and memories. Furthermore, they can also express sequences of novel trajectories that rats have never experienced, suggesting their possible involvement in navigational planning and the active construction of cognitive maps. Recent evidence also suggests that hippocampal replay sequences are able to represent the topological structure of complex environments (Wu & Foster, 2014). These sequences reflecting spatial structures can emerge rapidly after little experience. More recent findings demonstrate that rats can "preplay" spatial sequences during rest, even hours prior to exposure to the novel environment (Dragoi & Tonegawa, 2011). This suggests that rapid representations of novel sequences may arise from selection of pre-configured cell assemblies organised in a pre-existing temporal structure, rather than generated *de novo*. Using a model with pre-configured cell assemblies, we explore whether such a model can account for various properties of novel, forward and reverse replay sequences. We also use this model to make predictions about how these sequences can be used to build a cognitive map.

Disclosures: Y. Leow: None. **S.G. Lallée:** None. **C.Y. Tan:** None.

Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: ERC FP7 CIG 2013-618543

Title: Evoked responses in recurrent networks with multiple sub-populations

Authors: J. ALJADEFF[1], M. STERN[2], *O. BARAK[2];
[1]Neurobio., Univ. of Chicago, Chicago, IL; [2]Neurosci., Technion, Haifa, Israel

Abstract: We study the evoked responses of recurrent neural networks composed of multiple sub-populations of neurons ("cell-types") and a cell-type-specific connectivity rules. Recently we characterized the autonomous dynamics of such networks (Aljadeff, Stern, Sharpee, 2015). Significantly, the block structure of the connectivity matrix diverts the point of bifurcation from the quiescent state to a chaotic state, compared with networks with the same average connectivity strength but without structure (i.e. networks with a single cell-type). Furthermore, the autocorrelation structure of the global dynamics was more rich in the case of multiple sub-populations, compared with networks with a single population. These findings suggest that evoked responses would also depend on the cell-type-specific structure of the network, and not only on the average connectivity as in networks with a single cell-type. Previously it was shown for a network with a single population (and hence a single connectivity rule) that an the irregular spontaneous activity can be suppressed by a small amplitude input as long as its frequency is tuned to a band that depends on the average network gain (Rajan, Sompolinsky, Abbott, 2010). When the network is entrained by the stimulus, the intrinsic spontaneous dynamics are replaced with predictable correlated activity. In the network with multiple sub-populations, suppression of the spontaneous ongoing activity by an input is also observed. However the frequency to which the network is sensitive is now tuned by the parameters of the network: the size of each sub-population and the connectivity gain between every pair of sub-populations. If the connectivity gain in isolated regions of the network changes on a timescale slower than that of the spontaneous activity, for example through the effects of neuromodulation or neuro-glia interactions, this will change the properties of evoked responses in the entire network. The model studied by Rajan et al. is thought to be a step towards understanding the neural basis of stimulus and state-dependent attention. A limitation of this interpretation is that the stimulus that reduces the variability in the network (the "attended stimulus" that lies in a specific frequency band) is modified only if there are global changes to the gain in the network. By extending these results to networks with multiple sub-populations and characterizing their response properties we can develop an understanding of how local changes in a network can modulate the stimulus-

dependent suppression of variability, and therefore serve as a more realistic model of the neural basis of attention.

Disclosures: **J. Aljadeff:** None. **M. Stern:** None. **O. Barak:** None.

Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: CHIST-ERA; FWF Grant #I753-N23

European Union Project FP7-604102

Title: Stochastic network plasticity as Bayesian inference

Authors: ***R. LEGENSTEIN**, D. KAPPEL, S. HABENSCHUSS, W. MAASS;
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Abstract: Substantial experimental evidence (e.g. on spine motility, fluctuation of PSD-95 proteins) suggests that synaptic connections and synaptic efficacies are continuously fluctuating, to some extent even in the absence of imposed learning (see e.g. [1]). These findings raise the question how stable network function can be acquired and maintained in spite of these ongoing stochastic changes of network parameters. We present a novel conceptual framework for the organization of plasticity in neuronal networks in the brain that is based on stochastic variations of standard synaptic plasticity rules (e.g., Hebbian or STDP). The stochastic component of the plasticity rules continuously drives network parameters θ within a low-dimensional manifold of parameter space. This framework does not only explain how stable network function can be maintained in spite of ongoing parameter fluctuations, it also exhibits interesting new functional properties that have been posited from the perspective of learning theory [2, 3]. The low-dimensional parameter manifold represents a region where a compromise between overriding structural rules (such as sparse connectivity and heavy-tailed weight distributions) and good functional circuit properties is reached. Both structural plasticity [1] and synaptic plasticity can be integrated into this theory of network plasticity. This provides a theoretically founded framework for relating experimental data on spine motility to experimentally observed network properties. Furthermore, this framework endows neuronal networks with an important experimentally observed capability: Automatic compensation for network perturbations [4]. We

show that our alternative view can be turned into a rigorous learning model within the framework of probability theory. The low-dimensional parameter manifold can be characterized mathematically as the high probability regions of the posterior distribution of network parameters θ. More precisely, we propose that stochastic plasticity mechanisms enable brain networks to sample from this posterior, as opposed to the traditional view of learning as moving parameters to local optima θ* in parameter space. We demonstrate the advantages of this new theory in several computer simulations. These examples demonstrate how functional demands on network plasticity, such as incorporation of structural rules, automatic avoidance of overfitting, and inherent compensation capabilities, can be accomplished through stochastic plasticity rules.
[1] Holtmat A & Svoboda K. Neuron 2006; 49 [2] MacKay DJ. Neural Comp 1992; 4 (3) [3] Pouget A et al. Nat Neurosci 2013; 16 (9) [4] Marder E. PNAS 2011; 108 (3)

Disclosures: **R. Legenstein:** None. **D. Kappel:** None. **S. Habenschuss:** None. **W. Maass:** None.

Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH-MH60163

T32 NS058280-04S1

Title: Temporal scaling in functionally feedforward recurrent neural network models

Authors: ***N. HARDY**[1], D. V. BUONOMANO[1,2];
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Abstract: The ability to encode time and generate timed responses is essential to learning, sensory perception, and motor control. A well-known feature of motor control is the ability to produce motor patterns, such as reaching, typing, and speaking, at a range of different speeds (temporal scaling). Though a number of models address how the brain times simple events, few have addressed how complex spatiotemporal motor patterns are generated and how such patterns could undergo temporal scaling. Previous work based on standard firing-rate recurrent neural networks (RNNs) (Sompolinsky et al., 1988; Jaeger and Haas, 2004) along with a recently described recurrent learning rule (Laje & Buonomano, 2013) has shown that the dynamics (the

neural trajectories) of recurrent networks can robustly encode time. Here we show that these neural trajectories can also account for temporal scaling. Specifically, RNNs can be trained to reliably complete the same autonomously generated trajectory at different speeds (i.e. trajectory durations). This is accomplished by delivering a constant tonic input speed signal that governs the speed of the current trajectory. Once trained, the network can generalize the speed of its dynamics in response to untrained speed signals. During temporal scaling training, the network converges to states in which very similar "parallel" trajectories in neural space are generated at different speeds. Because of the severe dimensionality reduction of the RNN patterns at the level of the output units, the output patterns are virtually indistinguishable other than the fact that they are temporally warped. Experimental results have shown that the neural trajectory of a population of neurons can encode time. These trajectories are often a functional feedforward sequence of activity (Hanloser 2002; Long et al, 2010; Paton, 2015). Here we establish that RNNs based on more realistic firing rate units (where activity levels are bounded between 0 and 1, and with distinct excitatory and inhibitory populations), can generate multiple distinct functional feedforward trajectories embedded within the same network and triggered by different stimuli. Importantly, these networks also exhibit temporal scaling and generalize to novel speeds. Our results describe a general computational framework for reliably producing, and temporally rescaling spatiotemporally complex and biologically relevant neural trajectories_thus capturing an unaddressed but fundamental feature of motor control. These findings have implications for the problem of temporal warping of motor patterns as well as the neural mechanisms underlying the generation of complex motor patterns.

Disclosures: **N. Hardy:** None. **D.V. Buonomano:** None.

Poster

094. Computation: Other

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Topic: G.06. Computation, Modeling, and Simulation

Support: Sandia Hardware Acceleration of Adaptive Neural Algorithms Grand Challenge LDRD

Title: Dimensionality reduction and extrinsic modulation of cortical spiking networks

Authors: ***J. B. AIMONE**[1], C. E. WARRENDER[1], C. D. JAMES[2];
[1]Data-driven and Neural Computing, [2]Bio/Chem/Physical Microsensors, Sandia Natl. Labs., Albuquerque, NM

Abstract: Motor control and decision making regions of the cortex present a distinct challenge relative to sensory regions in that their inputs are less understood and their behavior is dominated by complex intrinsic dynamics. In recent years, *in vivo* characterization of these networks has benefited from analysis methods that investigate the population as a whole. These experimental studies have dovetailed with computational studies of intrinsically active balanced cortical network models. We describe how applying dimensionality reduction techniques, such as principal components analysis (PCA), to simulated cortical spiking networks can be used to describe the relationship between cortical networks and their behaviors. The recurrence and non-linear dynamics of cortical networks often makes their behavior borderline chaotic, and subtle changes to simulation conditions can make behavior of otherwise identical networks dramatically different. As a result, many modeling studies rely on statistical characterizations, such as ISI distributions, to compare the behavior of different networks. By using dimensionality reduction approaches to separate the temporal dynamics (e.g., PC trajectories) from the underlying behavioral manifold (e.g., PC basis sets), we can begin to directly compare the behavior of two networks that are parametrically varied. We first make a simple demonstration of this technique's utility by showing how some sources of noise, such as probabilistic synapses, preserve the underlying basis set; whereas forms of noise directed at network structure, such as synaptic weights, alter this basis set. We then use this technique to investigate how inputs interact with the intrinsic dynamics governed by the connectivity structure, and how outputs could separate input-driven dynamics from baseline dynamics. Finally, we use this technique to show how neuromodulation could result in dramatically different coding, greatly expanding the potential representational capacity of these networks. This approach could potentially help explain the interaction of internal and external dynamics during decision making and memory tasks in higher cortices.

Disclosures: **J.B. Aimone:** None. **C.E. Warrender:** None. **C.D. James:** None.

Poster

095. Computation: Networks and Experimentation

Location: Hall A

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Program#/Poster#: 95.01/BB76

Topic: G.06. Computation, Modeling, and Simulation

Support: NIH Pioneer DP1EY024503

Title: Automatic identification and classification of Hydra behaviors

Authors: *S. HAN, E. TARALOVA, R. YUSTE;
Biol. Sci., Columbia Univ., New York, NY

Abstract: Animal behaviors have been studied for centuries, but there is still a lack of efficient methods to automatically identify and classify all behaviors of an animal. Studies of animal behavior have been limited by the subjective and imprecise nature of human analysis, the limitation of the properties of human visual system and the slow speed of annotating behavioral data. Moreover, none of the major animal models allows the simultaneous observation of behavior and the complete neural activity from all neurons. Our group recently established a Hydra model with neuronal transgenic GCaMP6s, which allows the imaging of all neural activities while the animal is behaving. Here I have developed an automatic behavior identification and classification method for Hydra using machine learning approaches. I recorded behaviors from freely moving Hydra, extracted motion and shape features from the videos, and constructed a dictionary of these features. I identified behavior types using unsupervised clustering methods based on the dictionary, and trained classifiers for these behavior types. This method provides the opportunity of mapping the behaviors of Hydra and linking them to the neural activities. During evolution, Hydra is among one of the first species that developed a nervous system accompanied with a limited yet complex repertoire of behaviors. Studying the behaviors and the underlying neural networks of Hydra provides a unique opportunity of understanding the most basic rules of how nervous system compute and organize behaviors.

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Poster

095. Computation: Networks and Experimentation

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Topic: G.06. Computation, Modeling, and Simulation

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Title: Deep convolutional neural network inspired by development of visual acuity in Infants

Authors: *J. JUN, H. CHOI, J.-C. PARK, Y. JANG, D.-S. KIM;
KAIST, Daejeon, Korea, Republic of

Abstract: Deep learning is motivated by intuitions from structural and functional characteristics of the brain. Recent deep learning approaches such as convolutional neural networks (ConvNets) are breaking the records in various computer vision tasks especially large-scale Image recognition. After Convnet's success on the ImageNet Large-Scale Visual Recognition Challenge (ILSVRC) in 2012, various kinds of ConvNet architectures, learning methods and efficient regularization techniques have been introduced to improve their performance. Nonetheless, the gap between humans and machines is still large. Developmental studies of human's vision show that the human visual system rapidly develops after birth, especially in the first few years. Newborns only can detect changes in brightness or distinguish between stationary and moving objects, and their visual acuity is 12 to 25 times worse than a normal adult. After this period, the visual acuity starts to improve. In contrast, deep convolutional neural networks do not change the spatial frequency of input data during the training period. In this study, we hypothesized that the gradual change in spatial frequency of input data during the training period of ConvNets may decrease the overall training time. To this end, we utilized state of the art architecture of ConvNets for our subsequent experiments. The network consists of a number of convolution and pooling layers, followed by two fully-connected layers and a softmax layer. In order to measure the decrease in training time, we used CIFAR-10 image dataset to train our ConvNets. During training, we used input with fix-sized of 24 X 24 RGB image. At each training epoch, we gradually changed the resolution of the training images and passed the transformed data through the network. As a result, the trained model showed 91% classification accuracy on the test data. Additionally we found out that the overall training time was reduced compared to the conventional training method.

Disclosures: **J. Jun:** None. **H. Choi:** None. **J. Park:** None. **Y. Jang:** None. **D. Kim:** None.

Poster

095. Computation: Networks and Experimentation

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Topic: G.06. Computation, Modeling, and Simulation

Support: NHLBI Grant 5P01HL046925-17

Title: Generation of age-specific atlases for the segmentation of pediatric brains from magnetic resonance images

Authors: ***A. J. METZGER**[1], A. BENAVIDES[2], V. MAGNOTTA[3], P. NOPOULOS[2];
[2]Psychiatry, [3]Radiology, [1]Univ. of Iowa, Iowa City, IA

Abstract: Quantifying tissue volumes and surface areas of the brain from magnetic resonance (MR) images during late neonatal and early postnatal development can provide valuable insight into genetic and environmental influence on brain maturation. To detect consistent differences between healthy and abnormal subjects, population studies require accurate processing of a large number of samples. Thus, the tools for the analysis of these images need to be automated. Many of the tissue classification and segmentation tools used to analyze neuroimaging data rely on anatomical priors which provide the probability of a voxel's tissue type based on spatial location. However, these likelihoods are typically based on data from adult subjects. The goal of this project is to develop three age appropriate atlases (neonatal, 1 year old, and 2 year old) that account for the rapid growth and maturational changes that occur during this period. Tissue maps from this age group were initially created using an expectation maximization (EM) algorithm and an adult atlas and manually correcting the resulting tissue maps. To minimize the required manual corrections, the adult atlas was registered to the pediatric scans using high-dimensional, symmetric image normalization (SyN) registration. The subject images were then mapped to an age specific atlas space, again using the SyN registration, and the resulting transformation applied to the manually corrected tissue maps. The individual maps were averaged in the age specific atlas space and blurred to generate the anatomical priors. The resulting anatomical priors were then used by the EM algorithm to re-segment the initial training set as well as an independent testing set. The EM algorithm classified each voxel into one of ten possible tissue types: cerebral spinal fluid, cortical gray matter, white matter, corpus striatum, globus pallidus, thalamus, hippocampus, cerebellar white matter, cerebellar gray matter, and venous blood. An atlas based on four pediatric subjects provided superior results as compared to the adult atlas. The new atlas was improved by incrementally segmenting more subjects, manually correcting those segmentations, and adding the corrected tissue maps to the collection used to create anatomical priors. The age-specific atlases were created to provide quantitative volumetric data to study developmental differences between infants born pre- and full-term. Future studies utilizing these atlases might look to make progress towards determining onset and progression of disease, effectiveness of treatment, and/or risk factors in a wide variety of pediatric neurological conditions.

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Poster

095. Computation: Networks and Experimentation

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH/NIDCD R01DC011805

NIH/NIDCD R00DC009629

Title: The effect of task complexity on the stability of functional network communities

Authors: *S. FUERTINGER[1], K. SIMONYAN[2];
[1]Dept. of Neurol., Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Neurol., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Analysis of brain network communities provides important insights into the architecture of large-scale functional networks during a range of behaviors. However, questions arise whether modular network organization is stable across individual networks and whether it represents a characteristic signature of a specific behavior. To address these questions, we examined the stability of modular network partitions using community detection analyses of fMRI data acquired during four different conditions of increasing complexity, including the resting state, sequential finger tapping, meaningless syllable production, and meaningful speaking. In 14 right-handed monolingual English speaking healthy subjects, a 212-region whole brain parcellation was used to extract regionally averaged time-series, and zero-lag Pearson correlation coefficients were computed to construct weighted undirected graphs. Optimal modular network partitions were calculated by iteratively applying a heuristic community detection strategy. Partition distance (Pd) was quantified by calculating the variation of information between community affiliation vectors. The optimal partition of the group-averaged network for each condition was used as reference, and the stability of communities was assessed by performing a leave-one-out analysis. We found that resting-state formed six communities in the group-averaged network and showed a high degree of consistency within leave-one-out samples (Pd = 0.1±0.06, module N = 6.01±0.07) and high similarity to the group-averaged partition (Pd = 0.01±0.07). Task-related networks formed 5 communities during tapping, 4 communities during syllable production and 6 communities during speaking, all of which were stable across leave-one-out samples [module N = 5.2±0.73 (tap), 3.7±0.63 (syllable), 5.4±0.76 (speech)]. However, task-related networks exhibited somewhat reduced community consistencies with moderate deviations from the respective reference partitions [leave-one-out/group-averaged Pd = 0.16±0.05/0.20±0.03 (tap), 0.16±0.05/0.12±0.05 (syllable); 0.21±0.05/0.16±0.05 (speech)]. Our findings indicate that, while community architecture was specific to each examined condition, the stability of network communities was more sensitive to increased task complexity than to inter-subject variability.

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Poster

095. Computation: Networks and Experimentation

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NIH Grant R01MH10391002

Title: Time warping as a source of trial-to-trial neural variability

Authors: *P. N. LAWLOR[1], B. DEKLEVA[2], T. CYBULSKI[1], L. MILLER[2], K. KORDING[1]; [1]Physical Med. and Rehabilitation; Physiol., Rehabil. Inst. of Chicago, Northwestern Univ., Chicago, IL; [2]Physiol., Northwestern Univ., Chicago, IL

Abstract: One of the goals of modeling neural data is to identify and quantify types of variability. There are many such types, an important example of which is trial-to-trial variability: even when experimental conditions and behaviors are highly consistent, neural activity can vary considerably from one trial to the next. This is especially true for higher-order brain areas, where activity need not be tightly locked to sensory or motor events. For example, we can plan a movement, then choose when, and at what speed, to execute it. This observation motivates a type of modeling analysis that allows for neural responses with two key features: 1) variable offset in time between neural signals and external events, and 2) variable temporal scale allowing stretching and compression. To apply this idea to modeling, we have combined two well-known analysis tools: Generalized Linear Models (GLMs) and Dynamic Time Warping (DTW). GLMs have become a reliable analytical tool for neural data, but require the alignment of covariates to known or measured events in the real world. DTW provides a way to align two signals that differ by a warp (offset or scale), but lacks an underlying model of the signals being aligned. We combined the two by alternating between using DTW to “un-warp” the neural signal in each trial, and fitting the resulting un-warped signal with a standard GLM. We first applied this analysis framework to simulated single neuron activity generated from center-out reaching movements. The neuron’s spatial tuning curve followed cosine tuning and its temporal response varied between trials in offset and scale. Our approach correctly learned the warp for each trial, more accurately estimated the neuron’s tuning curves, and fit the data better than a GLM without time warping. We have also applied this framework to neural populations recorded from both macaque primary (M1) and dorsal premotor (PMd) cortex during a reaching task. Preliminary results suggest that time warping is present in both regions, and further, that the time warp for a

given trial is shared across neurons from the same region. For example, PMd activity related to reach target presentation varied in offset and stretch across trials, but was similar across neurons within a single trial. Future work will investigate whether accounting for time warp enables better decoding of behaviors such as reaching movements.

Disclosures: P.N. Lawlor: None. **B. Dekleva:** None. **T. Cybulski:** None. **L. Miller:** None. **K. Kording:** None.

Poster

095. Computation: Networks and Experimentation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 95.06/BB81

Topic: G.06. Computation, Modeling, and Simulation

Support: JSPS KAKENHI Grant Number 25870915

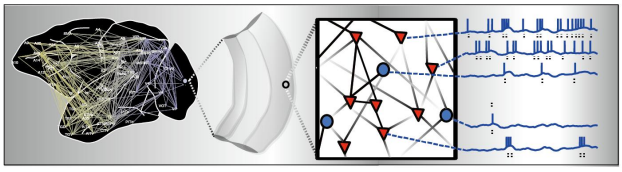
Grant-in-Aid for JSPS Fellows for Research Abroad

Title: Computer simulation of global brain dynamics at the neuronal resolution

Authors: *R. KOBAYASHI[1], M. SHIMONO[2];
[1]Natl. Inst. of Informatics, Tokyo, Japan; [2]Dept. of Physics, Univ. of Indiana, Bloomington, IN

Abstract: A key question in computational neuroscience is how microscopic components work together within the macroscopic brain scale. We demonstrate a computational model simulating the whole brain activity gathering neuronal components through columnar architectures (Figure). We used a Multi-timescale adaptive threshold model (Kobayashi et al., 2009) for the single neuron model, which is one of the most accurate models for reproducing the spike trains of a variety of cortical neurons *in vitro* (Gerstner and Naud, 2009). Before connecting through multiple brain regions, we tuned parameters about background inputs from subcortical nuclei and the ratio between excitatory and inhibitory synaptic strength on local neuronal circuits for sustaining activities within individual cortical regions (Potjans and Diesmann, 2012; Shimono and Beggs, 2014). We used a connectivity matrix provided from invasive tracing technique to sustain the accuracy, and especially the directionality of connectivity matrix (Deco et al., 2009; Shimono, 2013). Furthermore, the cortical network used in our study includes “weight” of connections (Markov et al., 2011). We reconstructed the number of neurons from the “weight”, and designed connections between neurons crossing different brain regions. The number of neurons at each brain region allowed us to integrate whole-brain network and neuronal dynamics

included in each brain region (Shimono, 2013). Computational modeling using non-invasive brain images is also important to use in cases of human brain structure (Izhikevich and Edelman, 2008). As summary, this computational model demonstrates a whole brain dynamics at the single neuron resolution for illuminating which parameter will be potentially critical to change the brain dynamics. Through the computer simulation, we will show a basis of understanding how optimally the brain structure is designed from the generated dynamics, including robustness against damages on the brain.



Disclosures: **R. Kobayashi:** None. **M. Shimono:** None.

Poster

095. Computation: Networks and Experimentation

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Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 95.07/BB82

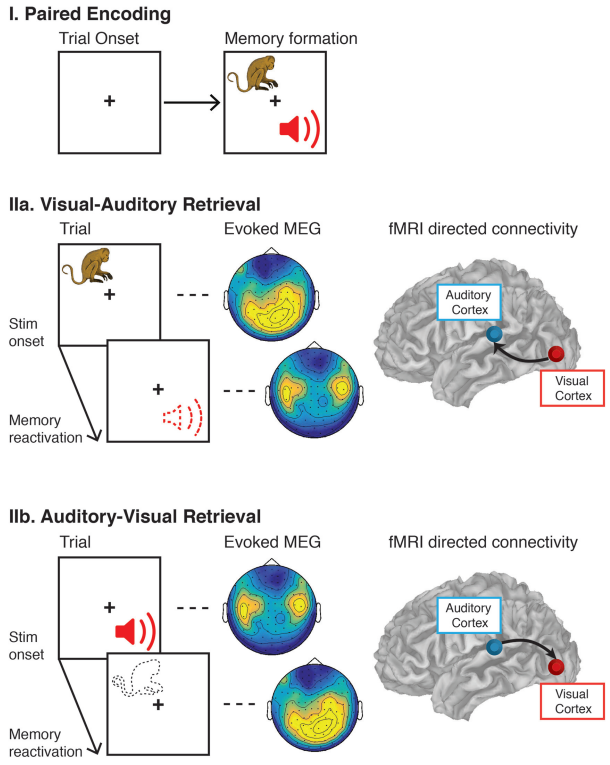
Topic: G.06. Computation, Modeling, and Simulation

Title: Exploiting sensory reactivation from memory to validate directed functional connectivity measures with fMRI and MEG

Authors: *R. D. MILL[1], A. BAGIć[3], W. SCHNEIDER[3], M. COLE[2];
[1]Ctr. for Mol. and Behavioral Neurosci., [2]Rutgers Univ., Newark, NJ; [3]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Mapping directions of influence within the human brain connectome represents the next phase in understanding its functional architecture. To this end, the efficacy of various methods of directed functional connectivity (also called effective connectivity) have been investigated in both the functional MRI (fMRI) and magnetoencephalography (MEG) literatures, primarily via recovering “ground truth” connectivity patterns embedded in simulated datasets. However, such approaches rely on numerous assumptions in the generative models used to simulate neuroimaging data. Hence, we explore a different strategy involving empirical data in which a ground truth directed connectivity structure could be anticipated with reasonable confidence. Specifically, we exploited the established “sensory reactivation” effect in episodic memory, in which the retrieval of sensory information leads to “reactivation” of regions

implicated in the perception of that sensory modality. We sought to implement a particularly powerful test by inducing a reversal in direction between visual and auditory brain regions across task conditions. Subjects underwent separate fMRI and MEG scanning whilst performing a paired associate task in which the onset of a visual stimulus cued retrieval of its auditory associate ("Visual-Auditory" condition), and the onset of an auditory stimulus cued retrieval of its visual associate ("Auditory-Visual" condition; see Figure). Task-evoked activation analyses of the fMRI and MEG data demonstrated reactivation in the auditory and visual cortices in the relevant retrieval conditions. Subsequent estimation of fMRI directed connectivity (via Patel's tau and IMaGES methods) recovered reversals in information flow in the anticipated directions, i.e. from visual to auditory cortices in the "Visual-Auditory" condition, and from auditory to visual cortices in the "Auditory-Visual" condition. These results complement simulation studies of directed connectivity, and begin to elucidate the dynamics that integrate brain regions during task performance.



Disclosures: **R.D. Mill:** None. **A. Bagić:** None. **W. Schneider:** None. **M. Cole:** None.

Poster

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Location: Hall A

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Program#/Poster#: 95.08/BB83

Topic: G.06. Computation, Modeling, and Simulation

Support: BBSRC Grant BB/L000814/1

BBSRC Grant BB/L002353/1

Title: The role of fasciculation in forming the connectome of the Xenopus tadpole spinal cord: a computational study

Authors: *R. BORISYUK[1], R. MERRISON-HORT[1], O. DAVIS[2,1], S. R. SOFFE[3]; [1]Plymouth Univ., Plymouth, United Kingdom; [2]Brighton and Sussex Med. Sch., Brighton, United Kingdom; [3]Univ. of Bristol, Bristol, United Kingdom

Abstract: The neurons in the developing spinal cord of a Xenopus tadpole form a neuronal network that is able to produce behaviours such as swimming and struggling from an early stage. We use computational models to shed light on which features of development are important for the formation of network that can produce patterns of neuronal activity that correspond to behaviour. Previously, we have shown that an axon growth model guided by gradients of chemical cues can produce axonal trajectories that closely match the available anatomical data [1]. Furthermore, when the synaptic connectivity derived from these generated axons is mapped onto a large scale physiological model, the resulting network responds to simulated “skin touch” input by generating a stable pattern of anti-phase rhythmic activity which resembles that seen in real tadpoles during swimming [2], as well as reproducing other experimental observations such as transient synchrony [3]. In this presentation we study the effects of adding axon fasciculation and repulsion mechanisms to our growth model. Axon fasciculation is a process whereby a developing axon can detect the presence of another nearby axon and begin to grow along the existing axon; repulsion is the opposite process, where axons actively avoid each other. Both fasciculation and repulsion have been observed in the growth of commissural axons in the spinal cord of very early stage tadpoles [4] and the computational model is used to investigate the possible function of these processes. The growth angle is adjusted to include either repulsion or fasciculation to the nearest axon. These additional mechanisms of axon growth result in dramatic changes to the pattern of axonal trajectories and connection architecture. References 1. Borisyuk R, Azad AK, Conte D, Roberts A, Soffe SR: A developmental approach to predicting neuronal connectivity from small biological datasets: a gradient-based neuron growth model. PLoS ONE 2014, 9(2): e89461 2. Roberts A, Conte D, Hull M, Merrison-Hort R, Azad AK, Buhl E, Borisyuk R, Soffe SR: Can simple rules control development of a pioneer vertebrate neuronal network generating behavior? J Neurosci 2014, 34(2): 608-621 3. Li W-C, Merrison-Hort R, Zhang H-Y, Borisyuk R: The Generation of Antiphase Oscillations and Synchrony by a Rebound-Based Vertebrate Central Pattern Generator. J Neurosci 2014, 34(17): 6065-6077 4. Moon M-S, Gomez TM: Adjacent pioneer commissural interneuron growth cones switch from contact avoidance to axon fasciculation after midline crossing. Dev Biol 2005, 288(2): 474-486

Disclosures: **R. Borisyuk:** None. **R. Merrison-Hort:** None. **O. Davis:** None. **S.R. Soffe:** None.

Poster

095. Computation: Networks and Experimentation

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Program#/Poster#: 95.09/BB84

Topic: G.06. Computation, Modeling, and Simulation

Title: How recurrent networks respond to complex stimulus sequences

Authors: ***A. P. PONZI**, J. R. WICKENS;
OIST, Okinawa, Japan

Abstract: Our ability to discriminate stimuli in predictable sensory streams improves with expectations of what stimuli may occur and when. Such streams entrain ongoing brain oscillations but how this happens and why it enhances discrimination is unclear. We previously showed [SFN 2014] that recurrent neural networks could generate anticipatory activity. We found that the neuronal and behavioural correlates of expectation emerge when weakly chaotic network oscillations are phase entrained by repetitive stimulus sequences. We show that stimulus presentations cause network phase resets. The network responses so generated are maximally discriminative when stimuli fall at their preferred phase in phase entrained networks. Discriminability increases continuously with both temporal regularity and stimulus type predictability. These factors also interact. Even random stimuli enhance target cue discriminability if streamed temporally regularly. Due to the gradual process of phase entrainment which occurs over multiple trials given stimuli show repetition dependent neuronal response while responses to unexpected oddball stimuli are large and distinct. Our results do not depend on specific network characteristics and are consistent with multiple streaming perceptual discrimination studies. Here we extend that study to investigate how networks respond to more complex types of hierarchical stimulus sequences such as occur in music, natural language and birdsong.

Disclosures: **A.P. Ponzi:** None. **J.R. Wickens:** None.

Poster

095. Computation: Networks and Experimentation

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Program#/Poster#: 95.10/BB85

Topic: G.06. Computation, Modeling, and Simulation

Support: NIDA DA018184

NIMH R01MH087660

NIGMS P20GM103472

Title: Secondary data analysis of sert knockout and wild-type mice using fastica to isolate active circuitry

Authors: *D. MCCOY[1], A. GONZALES[2], R. E. JACOBS[3], V. D. CALHOUN[4], E. L. BEARER[1];
[1]Pathology, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM; [2]Computer Sci., Univ. of New Mexico, Albuquerque, NM; [3]Beckman Inst. of the California Inst. of Technol., Pasadena, CA; [4]The Mind Res. Network, Albuquerque, NM

Abstract: New applications of independent component analysis (ICA) to mouse brain MR imaging data will provide pathologists and biochemists interested in tract-tracing methodologies with techniques to analyze active circuitry. This is the first report applying ICA analysis to manganese-enhanced magnetic resonance imaging (MEMRI) of living mouse brains. We acquired T_1 weighted imaging data at $90^3 \mu m^3$ isotropic voxel resolution, capturing images of the same mouse at different time points after Mn^{2+} injection. Here we report a secondary data analysis of results from Bearer et al. (2009), using group ICA via the GIFT toolbox (http://mialab.mrn.org/software/gift), which produces spatial maps and associated time course behavior. This approach determines the post-synaptic uptake of Mn^{2+} from the injection site in the medial prefrontal cortex in wildtype C57/b6 mice and same strain mice with the serotonin transporter (SERT) gene knockout. In our original report we performed Student's t-tests between knockout and wild-type mice to reveal SERT circuitry differences. Correlation between active circuits by ICA reveals functional connectivity of subcircuits within the time points. Results show isolation of injection, ventricular and transport networks. Localization of monoamine transportation networks can lead to development of better pharmaceutical drugs.

Disclosures: D. McCoy: None. **A. Gonzales:** None. **R.E. Jacobs:** None. **V.D. Calhoun:** None. **E.L. Bearer:** None.

Poster

095. Computation: Networks and Experimentation

Location: Hall A

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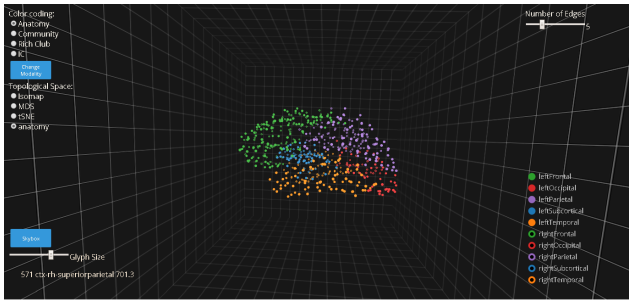
Program#/Poster#: 95.11/BB86

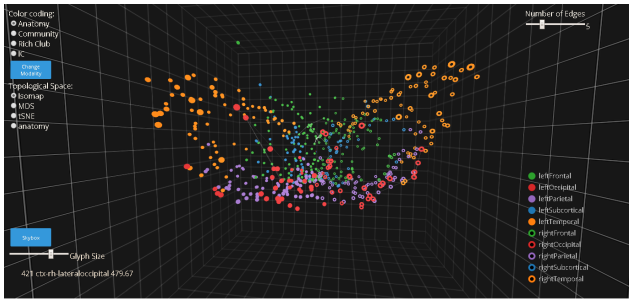
Topic: G.06. Computation, Modeling, and Simulation

Title: Braintrinsic: a virtual reality-compatible tool for exploring intrinsic topologies of the human brain connectome

Authors: *O. A. AJILORE[1], G. CONTE[2], A. YE[3], A. FORBES[4], A. LEOW[3];
[1]Univ. of Illinois-Chicago, Chicago, IL; [2]Computer Sci., [3]Psychiatry and Bioengineering, [4]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Thanks to advances in non-invasive technologies such as functional Magnetic Resonance Imaging (fMRI) and Diffusion Tensor Imaging (DTI), highly-detailed maps of brain structure and function can now be collected. In this context, brain connectomics have emerged as a fast growing field that aims at understanding these comprehensive maps of brain connectivity using sophisticated computational models. In this abstract, we present BRAINtrinsic, an innovative web-based 3D visual analytics tool that allows users to intuitively and iteratively interact with connectome data. Moreover, BRAINtrinsic implements a novel visualization platform that reconstructs connectomes' intrinsic geometry, i.e., the topological space as informed by brain connectivity, via dimensionality reduction. BRAINtrinsic is implemented with virtual reality in mind and is fully compatible with the Oculus Rift technology. Last, we demonstrate its effectiveness through a series of case studies involving both structural and resting-state MR imaging data.





Disclosures: **O.A. Ajilore:** None. **G. Conte:** None. **A. Ye:** None. **A. Forbes:** None. **A. Leow:** None.

Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: DYNAMO, Swiss National Foundation

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Title: Mechanisms underlying the modulation of motor patterns during epidural electrical stimulation of the lumbar spinal cord

Authors: ***M. CAPOGROSSO**[1], E. FORMENTO[1], E. MARTIN MORAUD[1], G. COURTINE[1], S. MICERA[1,2];
[1]Ecole Polytechnique Federale De Lausanne, Lausanne, Switzerland; [2]The Inst. of Biorobotics, Scuola Superiore Sant'Anna, Pisa, Italy

Abstract: Epidural electrical stimulation of lumbar segments induces locomotor states in animal models and humans with spinal cord injury. However, mechanisms underlying motor pattern formation during stimulation remain enigmatic. It has been hypothesized that EES restores the capacity of spinal networks to orchestrate locomotion after a lesion by increasing cell excitability. This excitation “tunes” the spinal circuitry to a state that enables the use of sensory information as the main source of control and coordination in the absence of supra-spinal inputs. Despite the wide acceptance of this theory, a clear mechanistic framework is still missing to explain how EES achieves this excitation, within which ranges this interpretation is valid and how to leverage this understanding to improve rehabilitation. In particular, EES recruits the same afferent pathways that are, in this context, presumably acting as the main source of control. This raises fundamental questions on the impact of EES on the signals that are driving locomotion, and on its capacity to synergistically interact with the dynamics that encodes gait-related information. To address these questions, we developed a realistic computational model of excitatory proprioceptive pathways in the spinal cord, and we studied the effect of stimulation parameters onto the natural activity of neural afferent, efferent, and muscular responses during

locomotion. We designed a realistic biological-inspired model of the spindles (Group Ia and II) reflex network of a couple of agonist-antagonist muscles encompassing a realistic model of alpha-Motoneurons, Ia inhibitory interneurons, group II excitatory interneurons, group Ia and group II afferents. The network receives inputs from EES by computing the induced firing rate in the recruited afferents using a FInite Element Model. In parallel, the natural firing rate of the afferents during stepping, is estimated by the use of a realistic biomechanical model of the rat hindlimb implemented in OpenSim. During different stimulation protocols, the modulation of rhythmic motor patterns emerged from the continuous changes in the state of the hindlimb. Thus, sensory information acted as a source of control that supported the elaboration of complex motor patterns to stand and step over a broad range of velocities. Based on these results, we designed model-driven stimulation strategies that controlled the amount and timing of muscle activity in real-time. These algorithms automatically corrected bilateral deficits of gait and balance in spinal cord injured rats. The present results may play an essential role in the translation of this intervention into clinical applications.

Disclosures: **M. Capogrosso:** None. **E. Formento:** None. **E. Martin Moraud:** None. **G. Courtine:** None. **S. Micera:** None.

Poster

095. Computation: Networks and Experimentation

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH NINDS NS035115

NIDCR DE022746

CIHR 301236

Title: Reliability of structural connectivity hubs in human brain network

Authors: ***E. VACHON-PRESSEAU**[1], S. BERGER[2], P. TÉTREAULT[2], A. APKARIAN[2], M. N. BALIKI[2];
[1]Northwestern Univ., Chicago, IL; [2]Northwestern university, Chicago, IL

Abstract: Hubs are regions that occupy central positions in large-scale brain networks and distribute information due to their high number of connections with other regions. The traditional degree-based approach identifies hubs based on the number of connections nodes has with other

nodes in the network. An alternative method characterizes hubs based on their connections within a same sub network as well as based on their participation across several sub networks of the brain. Using this methodology, we studied structural connections of large-scale brain networks and examined the stability of hubs across time. We collected diffusion tensor imaging (DTI) in 21 healthy controls to examine the structural topological properties of large-scale brain networks. Each subject was scanned 3 times every 6 months. Connectivity matrices were generated for each subject at each scanning session (n = 63) by parcelling the brain into 457 nodes and constructing networks from the edges between nodes representing white matter tracks. The connectivity matrices were further thresholded at a link-density ranging from 0.02 to 0.10 with incremental of 0.01 and then binarized. A common structural network was constructed from averaging the connectivity matrices at link-density of 0.05. Modularity analysis performed on connectivity matrices across all densities revealed the presence of 7 stable modules. Based on this modular organization, nodes were characterized by within-module degree Z-score and participation coefficient (a measure between 0-1 indicating between modules distribution of edges). 16 nodes were denoted as hubs (Z-scores > 2.5) and half of them displayed participation coefficient greater than 0.5. These hubs were mainly located in posterior insula and subcortical structures such as the thalamus and the basal ganglia. Normalized mutual information (NMI) was used to quantify the similarity between individual subject modularity and the mean group module structure at each scan. No effect of time was observed on NMI ($F(2,20) = 1.15$; $p = 0.33$) or within-module degree Z-score ($F(2,15) = 0.32$; $p = 0.66$) across scanning sessions. Previous studies have shown that hubs in functional networks of the human brain are sparse, located in the precuneus with low participation coefficient. Here, we show that brain network constructed form DTI structural connections displayed multiple subcortical hubs with high participation coefficients and are highly stable in time.

Disclosures: **E. Vachon-Presseau:** None. **S. Berger:** None. **P. Tétreault:** None. **A. Apkarian:** None. **M.N. Baliki:** None.

Poster

095. Computation: Networks and Experimentation

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Program#/Poster#: 95.14/BB89

Topic: G.06. Computation, Modeling, and Simulation

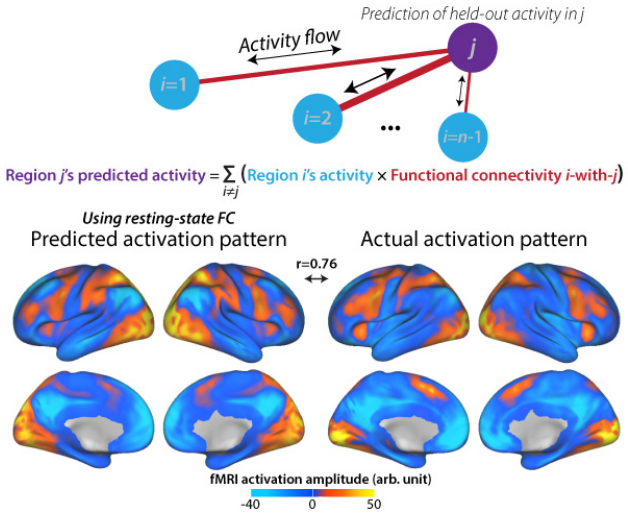
Support: NIH Grant MH096801

US Army Research Office grant W911NF-14-1-0679

Title: Intrinsic and dynamic functional network architectures shape task-evoked activation patterns in the human brain

Authors: *M. W. COLE[1], D. S. BASSETT[2], D. H. SCHULTZ[1];
[1]Rutgers Univ., Newark, NJ; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Investigations of brain function have largely bifurcated into focusing on either task activations or spontaneous brain activity correlations (resting-state functional connectivity). Recent evidence hints at a unification of these areas by demonstrating that task activation patterns correspond to resting-state functional connectivity patterns. However, the mechanisms underlying this correspondence remain unknown. We hypothesized that an intrinsic functional network architecture shapes activity flow among brain regions across both rest and task states. We tested this hypothesis in the human brain by modeling activity flow through functional networks, predicting each brain region's task activation as a combination of the task activations found in other brain regions. Predictions were accurate when resting-state functional connectivity governed the flow of activity. Predictions improved using task functional connectivity, suggesting dynamic updates to the brain's functional network architecture also shape task activation patterns. These results suggest activity flow through the brain's intrinsic functional network architecture provides a unifying framework for understanding activity across task and rest states, while dynamic modifications of that architecture refine activation patterns to allow implementation of task-specific functionality.



Disclosures: **M.W. Cole:** None. **D.S. Bassett:** None. **D.H. Schultz:** None.

Poster

095. Computation: Networks and Experimentation

Location: Hall A

Time: Saturday, October 17, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 95.15/BB90

Topic: G.06. Computation, Modeling, and Simulation

Title: A bidirectional interface for closed-loop hybrid neural systems

Authors: *F. D. BROCCARD[1], M. L. KHRAICHE[2], G. A. SILVA[2], G. CAUWENBERGHS[2]; [1]Inst. Neural Computation, [2]Bioengineering, UCSD, La Jolla, CA

Abstract: The coordination of distributed brain activity observed during many sensorimotor and higher cognitive processes largely depends on neuronal population activity. We propose an experimental and computational framework to investigate the dynamics of the interactions among functional neural networks. This framework is a closed-loop system composed of laboratory preparations of functional neuronal networks coupled to biologically realistic implementations of in silico neural networks implemented on software and/or biomimetic FPGA hardware. We present a proof of concept of this interface by establishing and maintaining a high level of synchronization between a biological network grown on a 256-channel multielectrode array and a spiking neural network model. The bidirectional interface may also provides a testbed for a new generation of neural prosthetics by allowing complex networks of any desired topology and level of complexity to be coupled with living neural tissue.

Disclosures: F.D. Broccard: None. **M.L. Khraiche:** None. **G.A. Silva:** None. **G. Cauwenberghs:** None.

Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: NRF-2010-0018837

NRF-2010-0012185

This research was supported by sk telecom

Title: Variational auto-encoder with convolutional neural network for complex image classification task

Authors: *Y. JANG, H. CHOI, J.-C. PARK, J. JUN, D.-S. KIM;
KAIST, Daejeon, Korea, Republic of

Abstract: These days deep learning have achieved much state-of-the-art performance in many machine learning tasks, such as image classification, object detection, speech recognition, and natural language processing. They utilize brain-like deep architecture consists of many layers of fully connected or convolutional layers to learn representation from the data to predict the correct answer for the specific target task. To train such deep architecture, recent deep learning models are largely dependent on labeled dataset, which is the pair of raw data and correct answer sets. However, these labeled dataset is scarce compared to unlabeled dataset, so in many tasks it is the bottleneck to develop human-level intelligence system. Furthermore, it is biologically implausible because human learns from the small volume of labeled data and mostly learn from unlabeled data. Many previous literatures have tried to overcome that limitation of learning systems by utilizing large volume unlabeled data like a human brain. However, most of them show severe performance degrade when labeled dataset is limited. In case of MNIST dataset, when we provide ten labeled examples per each digit best performance model gives 8.1 percent error rate. Meanwhile, variational auto-encoder records 3.33 percent of error rate. It is a breakthrough for the human-like learning system, which requires a small volume of labeled dataset to learn the appropriate representation of the given visual data. However, variational auto-encoder has its disadvantage. It is not trained well in case of networks with many layers so it cannot learn complex sets of images, such as large resolution general image classification task like ImageNet. Also, even it can use any kinds of neural network like convolutional neural network in principle, it cannot utilize such networks efficiently in practice. In this study, we suggest the model that learn from the large size of unlabeled dataset and small labeled dataset, which is similar to learning procedure in human. Our work uses the variational auto-encoder, but improve it by alleviating those limitations. To do that, we used convolutional neural network both in encoder and decoder parts of the variational auto-encoder. It gives more model capacity to learn more complex image dataset with fewer parameters. To test the effectiveness of our model, we perform two experiments on MNIST, CIFAR-10, and CIFAR-100 dataset. We perform image generation task to see that our model learns the appropriate generative model for various image dataset. Also, we perform image classification task. Our model shows reasonable performance improvement for semi-supervised learning setting.

Disclosures: **Y. Jang:** None. **H. Choi:** None. **J. Park:** None. **J. Jun:** None. **D. Kim:** None.

Poster

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Program#/Poster#: 95.17/BB92

Topic: G.06. Computation, Modeling, and Simulation

Support: NIH intramural research program

Title: Stability of latency and topology in functional networks of the human neo-cortex

Authors: *J. CHAPETON, S. INATI, K. ZAGHLOUL;
NINDS, NIH, Bethesda, MD

Abstract: Perhaps the most important step in analyzing functional brain connectivity is the initial construction of networks from electrophysiological or imaging data. Here, we construct stable functional networks of the human cortex based on electrocorticographic data acquired from epileptic patients undergoing seizure monitoring. Specifically, we calculate the average mutual information between the voltage traces from a pair of electrodes for a range of time lags, which allows us to extract the strength, latency, and direction of the connection between that pair. For each patient we construct networks using short time blocks collected over several days in order to examine the temporal evolution of these networks. We compare the connectivity at different time points by calculating measures of similarity between adjacency matrices and by comparing the networks' topological metrics. We find that the extracted network connectivity is stable over time scales ranging from minutes to days. Notably, connections that persisted over multiple time blocks had latencies that were remarkably similar across different days. Although many studies have shown that functional connectivity can change dynamically in a matter of seconds, our results suggest that there may be functional relationships that persist over much longer time scales.

Disclosures: **J. Chapeton:** None. **S. Inati:** None. **K. Zaghloul:** None.

Poster

095. Computation: Networks and Experimentation

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Title: Virtual slice in 3D: constructing and cutting a full-scale computational model of the dentate gyrus

Authors: *I. RAIKOV[1], C. J. SCHNEIDER[2], I. SOLTESZ[2];
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Abstract: The dentate gyrus is an integral part of the hippocampal formation that is thought to perform pattern separation of sensory inputs in order to facilitate context-specific encoding necessary for the formation and retrieval of memories (Neunuebel et al., J Neurosci. 2012; Leutgeb et al., Science 2007). We present a flexible and general computational methodology for the construction and functional simulation of neuroanatomically-detailed neuronal networks and its application towards a full-scale model of the rat dentate gyrus as well as slices of arbitrary thickness and position derived from it. The ability for automated slice model construction allows the modeling of reduced size networks without the need for synaptic weight scaling. Furthermore, it allows for a more direct comparison with the broad range of experimental results available for slice preparations of the dentate gyrus. The model incorporates a number of improvements over previous dentate modeling work. Most prominently, each granule cell has a computer-generated heterogeneous dendritic morphology in a realistic volume constrained by neuroanatomical reconstructions, using methodology previously described by the authors (Schneider CJ, Cuntz H, Soltesz I, PLoS Comput Biol. 2014 10(10):e1003921), with biophysical properties that agree closely with recent experimental literature and previous modeling studies. In addition, the model incorporates three new classes of interneurons, septotemporal density distributions of cells based on experimental measurements, and updated distributions of synaptic and electrical connectivity based on recent electrophysiological evidence. This work provides a powerful methodology for addressing the fundamental challenge of understanding how the specific morphological and spatial features of neurons relate to the function of whole regions of the brain. The flexibility and great increase in spatial resolution afforded by our methodology allows such questions to be investigated on multiple scales, from neurites and spines to circuits and systems.

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Poster

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Topic: G.06. Computation, Modeling, and Simulation

Title: Diffusion-based connectivity of the thalamus to the default-mode network

Authors: *S. I. CUNNINGHAM[1], D. TOMASI[1], N. D. VOLKOW[2];
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Abstract: Neuroimaging studies have identified functional interactions between the thalamus and default mode network (DMN) in studies of mindfulness, disorders of consciousness, and anesthesia-induced unconsciousness. However, less is known about the structural connectivity of the thalamus to regions within the DMN. In this study, we used diffusion tensor imaging to segment the thalamus based on its probabilistic connectivity to 6 DMN regions of interest (ROIs). Data from 37 healthy control subjects (17 males/20 females, ages 23-35 years) was obtained from the Human Connectome Database. Diffusion-weighted data was acquired in a Siemens 3T Connectome Skyra (TR/TE/flip angle = 5.52s/89.5ms/78º) through 6 runs representing 3 different shells of b = 1000, 2000, and 3000s/mm2 and processed using FSL's FDT diffusion toolbox. Six ROIs were selected from the dorsal DMN, including: the angular gyrus (AG), hippocampus, midcingulate cortex (MCC), medial prefrontal cortex (mPFC), superior frontal gyrus (sFG), and precuneus. Parcellation maps for each subject were created using probabilistic tractography to determine the number of connections from each voxel of the thalamus that had a 50% chance or greater of reaching each ROI. Each voxel of the thalamus was then assigned to the ROI that received the highest number of connections from that voxel. Combined results from 37 subjects revealed a highly symmetrical representation of the DMN across both hemispheres of the thalamus. The AG, hippocampus, mPFC, sFG, and precuneus all had a statistically similar number of connections to both hemispheres of the thalamus (p's > 0.05), whereas the MCC was more largely represented on the right thalamus ($p = 0.0001$). When compared to thalamic subdivisions defined in the Oxford Thalamic Connectivity Probability Atlas, the location of each DMN segment followed known structural connectivity of the thalamus to the cortex as a whole: thalamus voxels projecting to the mPFC, sFG, and MCC were primarily contained in the thalamic subdivision that projects to the pre-frontal cortex (p's < 0.05); precuneus and AG connectivity was mainly associated with the thalamic subdivision that projects to the posterior parietal lobe (p's < 0.01); and thalamus voxels projecting to the hippocampus were primarily located in a subdivision that projects to the temporal lobe. These findings suggest that a white matter network exists between the thalamus and DMN that follows known thalamocortical projections. Future comparison of thalamic structural connectivity with functional connectivity to the DMN will help determine if this underlying structure helps facilitate functional involvement of the thalamus in the DMN.

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Topic: G.06. Computation, Modeling, and Simulation

Title: Neuronal wiring specificity within and across connectomes

Authors: *M. BERNING, M. HELMSTAEDTER;
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Abstract: The computational capabilities of neuronal circuits are constrained by their structure. The degree to which circuit structure contributes to neuronal circuit function is unknown. One key open question is how much synaptic circuit structure varies between individuals. Obtaining constraints on this question is thus a key goal, but has been prohibitively expensive so far, since mapping even single local connectomes has consumed decades worth of work. Here we report the mapping of a second local connectome from mouse retina. We find that even rare cell types are preserved across individuals, that previously unknown connectivity rules between cell types are preserved, and we quantify the inter-individual single-cell wiring specificity in visual channels. This work provides quantitative bounds for the inter-individual variability of connectomes in the mammalian brain and provides a strong indication of high wiring specificity in the nervous system.

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Poster

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Title: Incremental learning of deep neural networks mitigating catastrophic forgetting

Authors: *H. CHOI, J. JUN, Y. JANG, J.-C. PARK, D.-S. KIM;
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Abstract: For neural network models and other machine learning systems, catastrophic forgetting has been a serious problem for many years. When a network is trained on one task and then trained on the other, it tends to forget previously learned knowledge while obtaining new information from the second task. According to previous studies in human learning systems, the neocortical learning algorithm is robust to catastrophic forgetting; it continuously replays memories stored in the hippocampus and strengthens the tasks that are not recently performed. This complementary algorithm enables human beings to learn new tasks and concepts in online fashion while standard neural network models are not suitable for such online and incremental learning. In this work, we investigate the possibility of incremental learning of deep neural networks by mitigating the problem of catastrophic forgetting. The baseline model architecture is deep convolutional networks that are known to perform well on various computer vision tasks, and we use dropout as our regularization method. We propose two key ideas to apply incremental learning into deep neural networks. First, when new data comes into the network, the network is cloned into two different networks, one with increased capacity and the other with the same size. After a reasonable amount of training, we compare the performance of two different networks and select the one that performs better. This adaptive scaling mechanism enables the model to be more robust to catastrophic forgetting since it learns to find the optimum network capacity with respect to the amount of data. As a result, the network can naturally learn new things incrementally with less information loss. The second approach is to reformat the sequence of input data that goes into the learning system. Just like human learning, we first train the network with more easily recognizable examples, and continuously increase the complexity of data by showing a more challenging sequence of examples to the model. This curriculum learning approach also enhances the learning capacity of the network, therefore helping the model to learn things in incremental way. To test the robustness of the proposed learning algorithm to catastrophic forgetting, we perform two different experiments with increasing complexity. We start with a simple task, recognizing the MNIST handwritten digits, and then increase the difficulty to CIFAR-10 dataset. The adaptive incremental learning algorithm showed reasonable performance increase compared to standard gradient-based training algorithm.

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Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: Grant 601215, EU FET Proactive, Call ICT-2011.9.11: Neuro-Bio-Inspired Systems (NBIS)

Title: Si elegans - An open access neurocomputational platform for testing behavioral paradigms in *C. elegans*

Authors: *A. BLAU[1], K. APPIAH[3], F. CALLALY[4], A. COFFEY[4], G. EPELDE[5], L. FERRARA[2], F. KREWER[4], P. LEŠKOVSKÝ[5], P. MACHADO[3], B. MC GINLEY[4], M. MCGINNITY[6,3], F. MORGAN[4], A. MUJIKA[5], A. PETRUSHIN[2], J. WADE[6];
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Abstract: Despite being one of the five best characterized model organisms with all of its 302 neurons and almost its entire connectome precisely mapped, there is only sparse knowledge on how the Caenorhabditis elegans nervous system codes for its rich behavioral repertoire. The European Si elegans project aims at unravelling *C. elegans*‘ nervous system function by its hardware emulation and its biophysically accurate embodiment in a virtual behavioral arena. We present the final platform implementation strategy and the recent system integration steps with special focus on the user-friendly neural response model generation and behavioral experiment definition tools. We describe the unique features of the neural representations by custom-designed field-programmable gate array (FPGA) boards synaptically communicating through an electro-optical connectome. Each of the 302 FPGAs can be configured with a *C. elegans*-specific neural response model. Both user-friendly and expert model definition tools (e.g., LEMS-based, import/export from existing neural libraries) are at the user’s disposition. The hardware nervous system controls the behavior of a virtual representation of the nematode in a virtual arena. The physics-based simulation will allow scientists to test both published and hypothetical behavioral paradigms just as in a real laboratory environment. Users will have access to all biologically relevant variables to study the neural events governing a certain behavior. We furthermore will explain how users can actively contribute to the development of add-on functionality of the platform in a peer-participation approach. For further information, please visit www.si-elegans.eu.

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Poster

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Topic: G.06. Computation, Modeling, and Simulation

Title: Computational modeling of the relationship between current dipoles and neural activity

Authors: *S. J. HESPRICH[1], S. A. BEARDSLEY[2];
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Abstract: Electroencephalography (EEG) is a commonly used technique to measure large-scale electrical activity in the brain in both clinical and research settings. Despite its widespread use, the precise relationship between EEG measured at the scalp and the neuronal activity generated within heterogeneous networks containing multiple cell types is not well understood. This study aims to look at how network activity relates to current dipole moments and extracellular potentials generated by populations of neurons. Anatomically accurate, multi-compartment neurons were used to simulate the dipole moments resulting from action potentials and post synaptic potentials (PSPs) across a variety of cell types. For each cell type, compartmental current dipole moments were summed across the cell to generate a dipole response function (DRF) for each neuron that combined the cell's anatomical configuration and electrical activity to create a point source representation of the equivalent current dipole for the cell. A thalamocortical network (Bazhenov et. al, 2002) was created using a combination of single and two compartment spiking neuron models to represent underlying network activity. In the model, 100 pyramidal and 25 interneurons, representing somatasensory cortex, and 50 thalamocortical and 50 reticular neurons, representing the thalamus, were used to simulate slow wave sleep patterns. Spiking activity from the network was convolved with the DRF for each neuron type and summed across the population. The resulting current dipole for the population was then used to compute extracellular potentials and compared to recordings of drug induced slow wave sleep in cats reported in the literature. Results suggest that bursting patterns of spiking activity in pyramidal cells contributes significantly to the neuron's current dipole. Direct contributions of action potentials to the net dipole moment generated across neurons are typically small due to

their short duration and corresponding limited temporal summation. During bursting neurons generate multiple action potentials that create an envelope in the DFR that lasts for the duration (approx 70ms) of the bursting activity. This longer envelope could enable a summative contribution of spiking activity across the population to the net current dipole. These insights could lead to additional understandings of the relationship between EEG signals and neural activity within heterogeneous networks.

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Poster

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Title: Intrinsic functional connectivity in valuation- and salience-sensitive networks provide a neural classifier for Autism Spectrum Disorder

Authors: *M. GHANE[1], J. A. RICHEY[2], R.-A. MÜLLER[3], K. T. KISHIDA[4];
[1]Virginia Polytechnic Inst. and State Universit, Blacksburg, VA; [2]Psychology, Virginia Polytechnic Inst. and State Univ., Blacksburg, VA; [3]Psychology, San Diego State Univ., San Diego, CA; [4]Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Research on autism spectrum disorder (ASD) shows evidence of early deficits in visual processing and increasing problems with social and non-social decision-making throughout development. One possible explanation for these deficits may be decreased connectivity in lower-level sensory processing networks or changes in connectivity between low- and high-order processing regions. Resting-state functional magnetic resonance imaging (rs-fMRI) is increasingly used as a way to characterize group differences between individuals with ASD and those who develop along typical trajectories (TD). These indices are often used to characterize group-level differences, which are descriptive and fail to characterize individual differences. We demonstrate that, per individual, pairwise correlations between a-priori defined regions-of-interest (ROIs) in valuation- and salience-sensitive networks can be used to accurately classify individuals with or without the diagnosis of ASD. We included rs-fMRI data (3T GE

scanner) from 42 ASD (*M age* = 13.90, SD = 2.68) and 44 TD (*M age* = 13.12, SD = 2.83) adolescents. Data were slice time and motion correction, scrubbing for artifacts, and co-registration to a standardized MNI-152 T1 weighted image. To assess intrinsic connectivity between regions within and across valuation- and salience-sensitive networks we produced a matrix of pair-wise correlations between 28 regions identified by peak task-based activation coordinates from Litt et al., 2011. The correlation coefficients per subject were transformed using Fisher's z-transformation and differences between the two groups (ASD vs. TD) assessed using two-sample t-test. Those connections that showed significant differences at $p<0.05$ (N=17 connections) were selected for further analysis. Next, we sought to train a cross-validated predictive model using the identified connections, additional demographic variables, and an automatic variable selection and model regularization approach called the "elastic net" (Zou and Hastie, 2005). We use this modern machine learning procedure to fit a penalized logistic regression model, which began with 17 connections plus variables for age, sex, and handedness. The resulting model included 15 of the original 17 suspected connections and did not retain age, sex, or handedness. The cross-validated, out-of-sample performance, of the reduced model gave an area under the curve of 0.8994 and sensitivity and specificity of 71.43% and 77.27%, respectively. The retained variables suggest that a purely neural account via resting state correlations is sufficient to achieve high discriminability between ASD and TD diagnoses.

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Poster

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Title: Context-dependent filtering as an emergent property of high dimensional networks

Authors: *M. S. GOLDMAN[1], K. R. ALLEN[2];
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Abstract: Context is an important factor for many aspects of cognition. Being able to attend to a single stimulus while filtering out other inputs has been a topic of great interest in both the experimental and theoretical literature. In a standard context-dependent decision making task, an animal is presented with two stimuli and a context signal indicating which of the two stimuli should be used to make a decision. An unresolved mystery for such tasks is how to reconcile the appearance of neural responses to distractor stimuli in the decision network with the fact that these stimuli do not significantly impact the integration of the target signal. Previous approaches have simply filtered out distractors before they reach the decision making circuit, or require networks with finely arranged separate attractors for each context. Our model only requires a single attractor, does not filter out distractors before they reach the decision making circuit, and works across a wide range of architectures. The critical observation upon which our results build is due to the statistics of high-dimensional spaces. In networks with large numbers of neurons, most patterns of neuronal firing will be orthogonal to each other. In the context of a decision making network in which evidence is accumulated along a line attractor, this means that high-dimensional input vectors will generically be orthogonal to the attractor direction. Thus, even if inputs arrive at the decision-making network and transiently cause neuronal responses, almost none of the input will be integrated unless the input vectors are specially arranged to align along the attractor. Therefore, filtering is the default operation of a large network, and a context signal must only act to rotate an incoming signal towards the attractor. Two stimuli can then be presented to the network, but only the one which is signaled by context will be integrated. This will not hold for small networks where the projection of a random distractor onto an integrating vector might be reasonably large. Here, the default operation is to integrate most inputs. We show this selective integration for a wide range of network architectures, with both linear and nonlinear neurons. Even for small rotations of the target signal, we see a drastic improvement in context-dependent classification accuracy when two competing stimuli are presented. These results provide a novel mechanism for context-dependent filtering in high dimensional networks. Furthermore, they illustrate how seemingly innocuous reductions to effective models with small numbers of units may discard critical network operations that require high dimensions.

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